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(54) Title: METHODS AND COMPOSITIONS FOR MODULATING CHOLINE ACETYLTRANSFERASE IN T CELLS

(57) Abstract: METHODS AND COMPOSITIONS FOR MODULATING CHOLINE ACETYLTRANSFERASE IN T CELLS
[00214] The present application is directed to methods and compositions for modulating choline acetyltransferase (ChAT) in T cells. The present application further provides methods of treating cancer and viral infection by modulating ChAT in T cells.

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METHODS AND COMPOSITIONS FOR MODULATING CHOLINE ACETYLTRANSFERASE IN T CELLS

CROSS REFERENCE TO RELATED APPLICATION

[0001] The present application claims the benefit of priority to U.S. Provisional Application No. 62/788,561, filed January 4, 2019, the contents of which is expressly incorporated herein in its entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] Although widely studied as a neurotransmitter, recent advances have revealed an important role for T-cell derived acetylcholine (ACh) in regulating immunity. However, the role of lymphocyte-derived ACh in viral infection is unknown.

[0003] The prototypic neurotransmitter acetylcholine (ACh) was the first neurotransmitter identified. ACh has numerous physiological roles, including mediating skeletal and smooth muscle contraction, communication between neurons, and induction of vasodilation. In addition to neurons, a population of CD4⁺ T cells and B cells express the enzyme choline acetyltransferase (ChAT), which catalyzes the rate-limiting step of ACh production. Although these ChAT-expressing T cells have a demonstrated impact on blood pressure and the release of inflammatory cytokines, the biological role of immune-derived ACh during infection has not been elucidated.

[0004] Tuning of the immune response can be of use in treatment of autoimmune disease or cancer. By dampening drivers of the inflammatory response, symptoms of autoimmune disease can be ameliorated or completely subdued. Manipulations of drivers of the immune response may also be used to treat cancer by inducing the body's own immune system to attack cancer cells.

[0005] There is a need for therapeutics capable of both fine-tuning the immune response and reaching the target tissue. These therapeutics can be used for treatment of both autoimmune disease and cancer.

SUMMARY OF THE INVENTION

[0006] In the examples described in greater detail below, it was shown that the enzyme choline acetyltransferase (ChAT), which catalyzes the rate-limiting step of ACh production, is robustly induced in both CD4⁺ and CD8⁺ T cells during lymphocytic choriomeningitis virus (LCMV) infection in an IL-21-dependant manner. Deletion of *Chat* within the T-cell compartment ablated vasodilation in response to infection, impaired the migration of anti-viral T cells into infected tissues, and ultimately compromised the control of chronic LCMV-CI13. The results revealed a genetic proof-of-function for ChAT in T cells during viral infection and identify a pathway of T-cell migration that sustains anti-viral immunity. In summary, the neurotransmitter acetylcholine is produced by T cells during viral infection to facilitate their entry into tissues and viral control.

[0007] In one aspect, the present invention provides a method for treatment of cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition modulating choline acetyltransferase in T cells.

[0008] In some embodiments, the modulating comprises increasing expression of choline acetyltransferase.

[0009] In other embodiments, the modulating comprises decreasing expression of choline acetyltransferase.

[0010] In some embodiments, the pharmaceutical composition modulating choline acetyltransferase in T cells comprises IL-21.

[0011] In other embodiments, the pharmaceutical composition modulating choline acetyltransferase in T cells comprises TGF- β .

[0012] In some embodiments, the modulating comprises increasing activity of choline acetyltransferase.

[0013] In other embodiments, the modulating comprises decreasing activity of choline acetyltransferase.

[0014] In some embodiments, the method additionally comprises administering one or more anti-cancer therapeutic agents. In further embodiments, the one or more anti-cancer therapeutic agents comprises at least one checkpoint inhibitor. In further embodiments, the at least one checkpoint inhibitor is a member selected from the

group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.

[0015] In some embodiments, the pharmaceutical composition modulating choline acetyltransferase in T cells and the one or more anti-cancer therapeutic agents are administered locally.

[0016] In other embodiments, the pharmaceutical composition modulating choline acetyltransferase in T cells and the one or more anti-cancer therapeutic agents are administered systemically.

[0017] In other embodiments, the pharmaceutical compound modulating choline acetyltransferase in T cells is administered locally and one or more of the one or more anti-cancer therapeutic agents is/are administered systemically.

[0018] In other embodiments, the pharmaceutical compound modulating choline acetyltransferase in T cells is administered systemically and one or more of the one or more anti-cancer therapeutic agents is/are administered locally.

[0019] In some embodiments, the pharmaceutical composition is conjugated to at least one of the one or more anti-cancer therapeutic agents.

[0020] In another aspect, the present invention provides a method for treatment of cancer to a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of T cells engineered to increase expression of choline acetyltransferase. In some embodiments, the T cells are autologous. In some embodiments, the T cells are antigen-specific.

[0021] In some embodiments, the method further comprises administering one or more anti-cancer therapeutic agents. In some embodiments, the one or more anti-cancer therapeutic agents comprises at least one checkpoint inhibitor. In some embodiments, the at least one checkpoint inhibitor is a member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.

[0022] In another aspect, the present invention provides a method for treatment of cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of a virus that is engineered to mediate induction of choline acetyltransferase. In some embodiments, the method further comprises administering one or more anti-cancer therapeutic agents. In some embodiments, the one or more anti-cancer therapeutic agents comprises at least one checkpoint inhibitor. In some embodiments, the at least one checkpoint inhibitor is a member

selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.

[0023] In another aspect, the present invention provides a method for the treatment of cancer in a subject in need thereof, the method comprising (a) obtaining a sample from the subject; (b) measuring the level of choline acetyltransferase present in the sample; (c) assessing that the subject has low choline acetyltransferase expression; and (d) administering a pharmaceutically effective amount of a vasodilator.

[0024] In some embodiments, method comprises measuring the level of choline acetyltransferase present in the sample by immunoassay.

[0025] In other embodiments, the method comprises measuring the level of choline acetyltransferase present in the sample by a polymerase chain reaction assay.

[0026] In some embodiments, the method comprises administering one or more anti-cancer therapeutic agents with the vasodilator. In some embodiments, the one or more anti-cancer therapeutic agents comprises at least one checkpoint inhibitor. In some embodiments, the at least one checkpoint inhibitor is a member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.

[0027] In some embodiments, the vasodilator and the one or more anti-cancer therapeutics is/are delivered locally. In other embodiments, the vasodilator and the one or more anti-cancer therapeutics is/are delivered systemically. In other embodiments, the vasodilator is delivered locally and one or more of the one or more anti-cancer therapeutics is/are delivered systemically. In other embodiments, the vasodilator is delivered systemically and one or more of the one or more anti-cancer therapeutics is/are delivered locally.

[0028] In some embodiments, the vasodilator is conjugated to at least one of the one or more anti-cancer therapeutic agents.

[0029] In some embodiments, the vasodilator is coated on T cells *ex-vivo* and the T cells coated by the vasodilator are administered to the subject.

[0030] In some embodiments, the vasodilator increases tumor infiltrating lymphocytes in a tumor.

[0031] In another aspect, the present invention provides a method for the treatment of cancer in a subject in need thereof, the method comprising (a) obtaining

a sample from the subject; (b) measuring the activity of choline acetyltransferase present in the sample; (c) assessing that the subject has low choline acetyltransferase activity; and (d) administering a pharmaceutically effective amount of a vasodilator.

[0032] In some embodiments, the activity of choline acetyltransferase present in the sample is determined by measuring relative levels of a member selected from the group consisting of acetylcholine, choline, and acetyl-CoA.

[0033] In some embodiments, the method comprises measuring the level of choline acetyltransferase activity present in the sample by an immunoassay and/or polymerase chain reaction assay.

[0034] In some embodiments, the method comprises administering one or more anti-cancer therapeutic agents with the vasodilator. In some embodiments, the one or more anti-cancer therapeutic agents comprises at least one checkpoint inhibitor. In some embodiments, the at least one checkpoint inhibitor is a member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.

[0035] In some embodiments, the vasodilator and the one or more anti-cancer therapeutics is/are delivered locally. In other embodiments, the vasodilator and the one or more anti-cancer therapeutics is/are delivered systemically. In other embodiments, the vasodilator is delivered locally and one or more of the one or more anti-cancer therapeutics is/are delivered systemically. In other embodiments, the vasodilator is delivered systemically and one or more of the one or more anti-cancer therapeutics is/are delivered locally.

[0036] In some embodiments, the vasodilator is conjugated to at least one of the one or more anti-cancer therapeutic agents.

[0037] In some embodiments, the vasodilator is coated on T cells *ex-vivo* and the T cells coated by the vasodilator are administered to the subject.

[0038] In another aspect, the present invention provides a method for treatment of cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective pharmaceutical composition comprising choline acetyltransferase.

[0039] In some embodiments, the cancer is melanoma. In some embodiments, the method or pharmaceutical composition increases tumor infiltrating lymphocytes in a tumor.

[0040] In another aspect, the present invention provides a method for the treatment of viral infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition modulating choline acetyltransferase in T cells. In some embodiments, the modulating comprises increasing expression of choline acetyltransferase. In other embodiments, the modulating comprises decreasing expression of choline acetyltransferase. In some embodiments, wherein the pharmaceutical composition modulating choline acetyltransferase in T cells comprises IL-21. In some embodiments, the pharmaceutical composition modulating choline acetyltransferase in T cells comprises TGF- β . In some embodiments, modulating comprises increasing activity of choline acetyltransferase. In other embodiments, modulating comprises decreasing activity of choline acetyltransferase.

[0041] In some embodiments, the method comprises additionally administering one or more anti-viral therapeutic agents.

[0042] In some embodiments, the pharmaceutical composition is conjugated to at least one of the one or more anti-viral therapeutic agents

[0043] In another aspect, the present invention provides a method for the treatment of viral infection in a subject in need thereof, the method comprising (a) obtaining a sample from the subject; (b) measuring the level of choline acetyltransferase present in the sample; (c) assessing that the subject has low choline acetyltransferase expression; and (d) administering a pharmaceutically effective amount of a vasodilator. In some embodiments, the method comprises measuring the level of choline acetyltransferase present in the sample by immunoassay.

[0044] In other embodiments, the method comprises measuring the level of choline acetyltransferase present in the sample by a polymerase chain reaction assay.

[0045] In some embodiments, the method comprises additionally administering one or more anti-viral therapeutic agents.

[0046] In some embodiments, the vasodilator is conjugated to at least one of the one or more anti-viral therapeutic agents.

[0047] In another aspect, the present invention provides a method for the treatment of viral infection in a subject in need thereof, the method comprising (a) obtaining a sample from the subject; (b) measuring the activity of choline

acetyltransferase present in the sample; (c) assessing that the subject has low choline acetyltransferase activity; and (d) administering a pharmaceutically effective amount of a vasodilator.

[0048] In some embodiments, measuring the activity of choline acetyltransferase present in the sample is determined by measuring relative levels of a member selected from the group consisting of acetylcholine, choline, and acetyl-CoA.

[0049] In some embodiments, the method comprises measuring the level of choline acetyltransferase present in the sample by immunoassay and/or polymerase chain reaction assay.

[0050] In some embodiments, the method comprises additionally administering one or more anti-viral therapeutic agents.

[0051] In some embodiments, the vasodilator is conjugated to at least one of the one or more anti-viral therapeutic agents.

[0052] In another aspect, the present invention provides a method for treatment of viral infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective pharmaceutical composition comprising choline acetyltransferase.

[0053] In some embodiments, the method or pharmaceutical composition increases viral clearance.

BRIEF DESCRIPTION OF THE DRAWINGS

[0054] FIG. 1A – FIG. 1L: **FIG. 1A and FIG. 1B** show ChAT-GFP⁺ and ChAT-GFP⁻ animals infected (or not, naïve) with LCMV-Arm and the expression of ChAT-GFP in total CD4⁺ (FIG. 1A) and CD8⁺ (FIG. 1B) T cells 8 days post-infection compared to uninfected ChAT-GFP⁺ cohorts. **FIG. 1C, FIG. 1D, and FIG. 1E** show the fraction of virus specific CD4⁺ (FIG. 1C) or CD8⁺ (FIG. 1D and FIG. 1E) T cells expressing ChAT-GFP in spleens of ChAT-GFP⁺ mice at 8, 20, and 30 days post-infection with LCMV-Arm or LCMV-CI13, identified by tetramer staining and flow cytometry. **FIG. 1F, FIG. 1G, and FIG. 1H** show representative plots of ChAT-GFP expression in the splenic CD4⁺ (FIG. 1F) or CD8⁺ (FIG. 1G and FIG. 1H) T cells from the mice in FIG. 1C to FIG. 1E at 30 days post-infection. **FIG. 1I and FIG. 1J** show quantitation of ChAT-GFP expression by Fas⁺ GL-7⁺ GC B cells or Fas⁻ GL-7⁻ non-GC B cells at the indicated time points post-infection with LCMV-Arm (FIG. 1I) or LCMV-CI13 (FIG. 1J). *Chat*-GFP expression was evaluated in Fas⁺ GL-7⁺ germinal center B cells (black) or Fas⁻ GL-7⁻ non-GC B cells (gray) at indicated time points post LCMV-Arm (FIG. 1I) or LCMV-CI13 (FIG. 1J) infection. Mean±S.E.M. Statistical significance determined by unpaired two-tailed t-test. Flow plots are representative data from 8 days post-infection. Composite or representative of at least two experimental cohorts, n=10-15 **FIG. 1K** shows spleens from either GFP-negative (left) or ChAT-GFP mice isolated 8 days post LCMV-Arm or LCMV-CI13 infection stained for GFP in formalin-fixed paraffin embedded sections by IHC, visualized at 10x, with brown staining indicating GFP and blue staining indicating nuclei. **FIG. 1L** shows ChAT expression in CD4⁺ and CD8⁺ populations from pooled splenocytes from ChAT-GFP mice infected 8 days previously with LCMV-CI-13. Pooled splenocytes from n=5 *Chat*-GFP mice infected 8 days previously with LCMV-CI-13 were sorted to obtain CD4⁺GFP⁺, CD4⁺GFP⁻, CD8⁺GFP⁺, and CD8⁺GFP⁻ populations. RNA was isolated from the cells, and expression of *Chat* and *Rsp9* was evaluated by RT-PCR in technical triplicates. *Chat* expression in CD4⁺ and CD8⁺ populations was normalized to the expression in the relevant sorted GFP⁻ population. Mean±S.E.M. Representative of 2 experimental cohorts.

[0055] FIG. 2A – FIG. 2H show *Chat* expression in virus-specific CD4⁺ T-cell, CD8⁺ T-cell, and B-cell subsets. **FIG. 2A-D** show ChAT-GFP mice infected with LCMV-Arm (FIG. 2A and FIG. 2B) or LCMV-CI13 (FIG. 2C and FIG. 2D) and LCMV-GP61 specific CD4 T cells were identified by tetramer staining and flow cytometry at the indicated time

points. **FIG. 2A** shows LCMV-GP61 specific CD4⁺ T cell expression of CXCR5 and PD-1 8 days post-LCMV-Arm infection. Populations were divided into CXCR5⁺ PD-1^{bright} (T_{fh}, red in original), CXCR5⁺ PD-1^{dim} (T_{mem}, green in original) and CXCR5⁻ PD-1^{dim} (T_{effector}, blue in original), and further evaluated for *Chat*-GFP expression. Two-way ANOVA was performed ($p < 0.0001$), followed by multiple comparisons between groups.

FIG. 2B shows the fraction of GP61-specific CD4⁺ T cells of each population (T_{fh}, T_{memory}, T_{effector}) expressing ChAT-GFP in FIG. 2A quantitated at 8, 20, and 30 days post-infection. Mean+S.E.M. **FIG. 2C** shows LCMV-GP61 specific CD4⁺ T cells at 8 days post LCMV-CI13 infection. **FIG. 2D** shows CD4⁺ GP61-specific T cells fractionated as in FIG. 2B, and evaluated at 8, 20, and 30 days post-LCMV-CI13 infection.

Representative or composite of at least two experimental cohorts. Arm D8 n=16; D20 n=7; D30 n=12. CI13 D8 and D20 n=9; D30 n=11. **FIG. 2E** shows D^b(GP33)-tetramer+ ChAT-GFP⁺ and ChAT-GFP⁻ cell expression of CD127 and KLRG-1. D^b(GP33)-tetramer⁺ *Chat*-GFP⁺ and *Chat*-GFP⁻ cells were gated and expression of CD127 and KLRG-1 was evaluated on each population. Representative of two experimental cohorts, n=6 *Chat*-GFP⁺, n=2 GFP⁻ controls. **FIG. 2F, FIG. 2G, and FIG. 2H** show MFI determination of levels of inhibitory receptors PD-1 (FIG. 2F), Tim-3 (FIG. 2G), and LAG-3 (FIG. 2H) in ChAT-GFP⁺ and ChAT-GFP⁻ D^b(GP33)-specific (left) or D^b(GP276)-specific (right) CD8⁺ T cells that were isolated from ChAT-GFP⁺ animals infected with LCMV-CI13 and evaluated at 8, 20, and 30 days post-infection.

[0056] FIG 3A – FIG. 3F: **FIG. 3A** shows the expression of *ChAT*-GFP⁺ and *ChAT*-GFP⁻ P14 CD8⁺ T cells stimulated in vitro with GP33 peptide and indicated cytokines for 5 days. The expression of *Chat*-GFP in the P14 cells was determined by flow cytometry. Composite of 2 experimental cohorts, mean+range min to max, n=5 *Chat*-GFP⁺ P14 mice. Significance tested using one-way ANOVA $p < 0.0001$, significance between samples determined by *t*-test depicted). **FIG. 3B** shows the fraction of indicated splenic populations expressing *ChAT*-GFP determined by flow cytometry in IL-21R^{+/+}, IL-21R^{+/-}, and IL-21R^{-/-} mice at 8 days post-infection. *Il21r*^{+/+} (black), *Il21r*^{+/-} (gray) and *Il21r*^{-/-} (white) mice expressing *Chat*-GFP were infected with LCMV-CI13, and *Chat* expression determined in splenocyte fractions 8 days post-infection by flow cytometry. Box and whiskers drawn with the Tukey method, line at median. Values outside of 1.5 IQR are depicted as individual symbols. Two-way ANOVA was performed ($p < 0.0001$), followed by multiple comparisons between groups. **FIG. 3C** shows MFI determination of GFP levels on ChAT⁺ virus-specific cells that were isolated from the

ChAT-GFP-expressing IL-21R^{+/+}, IL-21R^{+/-}, and IL-21R^{-/-} mice in FIG. 3B at 8 days post-LCMV-CI13 infection. **FIG. 3D** shows representative flow plots of virus-specific D^b(GP33)+ (upper) or D^b(GP276)+ (lower) CD8+ T cells in the ChAT-GFP-expressing IL-21R^{+/+} or IL-21R^{-/-} mice in (FIG. 1B) at 8 days post-infection. Representative of 2-3 experimental cohorts, n=8-13. Statistical significance determined by unpaired two-tailed *t*-test **p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001 **FIG. 3E** shows the fraction of the indicated splenic populations expressing ChAT-GFP determined by flow cytometry in Stat^{flox/flox} Cre- wild type ChAT-GFP+ or Stat3^{flox/flox} CD4-Cre ChAT-GFP+ mice infected with LCMV-CI13 at 8 days post-infection. **FIG. 3F** shows representative flow plots of virus-specific D^b(GP33)+ (upper) or D^b(GP276)+ (lower) CD8+ T cells in the ChAT-GFP-expressing Stat3^{WT} (Stat3^{flox/flox} Cre-) or Stat3 T cell^{KO} mice in FIG. 3E at day 8 post-infection.

[0057] FIG. 4A – FIG. 4I show loss of *Chat* in T cells compromises control of viral infection. **FIG. 4A and FIG. 4B** show numbers of D^b(GP33)+ (FIG. 4A) or D^b(GP276)+ (FIG. 4B) CD8+ splenocytes determined at the indicated time points by tetramer staining and flow cytometry. Mean±S.E.M. Composite of 3-4 experimental cohorts, n=10-16 **FIG. 4C and 4D** show expression of inhibitory receptors PD-1, Tim-3, and LAG-3 on D^b(GP33)+ (FIG. 4C) or D^b(GP276)+ (FIG. 4D) cells by flow cytometry. **FIG. 4E** shows a representative flow plot of PD-1 and Tim-3 expression by the D^b(GP33)+ CD8+ T cells in FIG. 4C at 30 days post-infection. **FIG. 4F** shows splenocytes from ChAT^{WT} and T-ChAT^{KO} animals stimulated with GP276 peptide *in vitro*, and the number of cells producing IFN γ , TNF α , and/or IL-2 quantified by intracellular cytokine staining. The fraction of the total D^b(GP276) specific cells which are non-functional was determined by comparing the number of cytokine-producing cells to the number of tetramer-binding cells. Of the functional cells, the fraction which are monofunctional or polyfunctional was determined. Mean of 2-3 experimental cohorts, D30 n=9, D60 n=8 **FIG. 4G and FIG. 4H** show MFI determination of T-bet expression in D^b(GP33)+ (FIG. 4G) or D^b(GP276)+ (FIG. 4H) splenocytes isolated from ChAT^{WT} or T-ChAT^{KO} mice at the indicated days post-infection. **FIG. 4I** shows viral titers in serum of ChAT^{WT} or ChAT^{KO} mice determined at the indicated time points by plaque assay. Viral titers in the serum of either *Chat*^{WT} (black) or T-*Chat*^{KO} (white) were determined at indicated time points by plaque assay. Each symbol indicates an individual animal. Composite data of 2-3 experimental replicates, n=11-23. Line at limit of detection, 333 pfu/mL.

[0058] FIG. 5A – FIG. 5H: **FIG. 5A and FIG. 5B** show quantitation of *in vivo* cytolytic activity in the spleen (FIG. 5A) and liver (FIG. 5B) of ChAT^{WT} or T-ChAT^{KO} mice at 8 days post-LCMV-CI13 infection. **FIG. 5C and FIG. 5D** show quantitation of numbers of virus-specific CD8⁺ T cells identified by intravascular staining, followed by conventional tetramer and CD8 staining *ex vivo*. **FIG. 5E** shows a schematic of experimental design for adoptive transfer of ChAT^{WT} P14 or ChAT^{KO} P14 T cells into either ChAT^{WT} or T-ChAT^{KO} recipient mice, which were subsequently infected with LCMV-CI13. **FIG. 5F** shows the ratio of number of P14 T cells migrated into tissues of the recipient mice in FIG. 5E to the number of endogenous Db(GP33)⁺ cells, as evaluated at 30 days post-infection. Mean±S.E.M. Composite of 2 experimental cohorts for controls and 4 experimental cohorts, n=5-10 control animals, n=12-17 experimental animals. Statistical significance for all samples determined by unpaired two-tailed *t*-test **p*<0.05, ***p*<0.01, ****p*<0.001 *****p*<0.0001 **FIG. 5G** shows the fraction of liver-infiltrating D^b(GP276)-specific CD8 T cells expressing granzyme B and the fraction of liver-infiltrating CD8 T cells expressing CD107a and IFN γ after *in vitro* stimulation with GP276 compared to the total number of liver-infiltrating D^b(GP276)⁺ cells. The fraction of liver-infiltrating CD8 T cells expressing CD107a and IFN γ after *in vitro* stimulation with GP276 was compared to the total number of liver-infiltrating D^b(GP276)⁺ cells to determine the percent of GP276-specific cells capable of degranulation. Mean±S.E.M., composite of 2 experimental cohorts n=11-12. **FIG. 5H** shows the analysis schematic for the P14 transfer experiments. Chat^{WT} P14 or Chat^{KO} P14 T cells were transferred into either Chat^{WT} or T-Chat^{KO} recipient mice, which were subsequently infected with LCMV-CI13. Vascular cells in different tissues were marked as in 30 days post infection. The D^b(GP33)-specific non-vascular cells were evaluated to determine the relative ratio of endogenous and P14 cells in the spleen and peripheral organs. The relative abundance of P14 cells in the organs was then compared to the spleen, to determine whether P14 cell migrated better, as well, or worse than the endogenous D^b(GP33)-specific cells.

[0059] FIG. 6A – FIG. 6G show vasodilation during infection is dependent upon Chat-expressing T cells and is critical for viral control. **FIG. 6A** shows blood vessel diameter measured in z-stacks obtained by 2-photon microscopy in ChAT^{WT} (gray) or T-ChAT^{KO} (white) liver at 8-9 days post-infection with LCMV-CI13. **FIG. 6B** shows representative images of blood vessels from the mice in FIG. 6A. **FIG. 6C** shows representative images of arterial trees in livers from ChAT^{WT} or T-ChAT^{KO} mice at 8 days post-infection with LCMV-CI13 after the mice were either gavaged with minoxidil

or injected with L-NAME on days 6, 7, and 8 post-infection. Scale bar is 200 μ M. **FIG. 6D** and **FIG. 6E** show pharmaceutical modulation of vasodilation in T-*Chat*^{KO} and wild type mice during infection. *Chat*^{WT}, T-*Chat*^{KO}, and C57Bl/6 mice were infected with LCMV-CI-13. Treated mice were either gavaged with minoxidil or injected i.p. with L-NAME on days 6, 7, and 8 postinfection. Arterial tree of the liver was perfused with radio-opaque Microfil 8 days post infection, and imaged using a microCT scanner at a resolution of 16 μ m. **FIG. 6D** shows quantification of number of terminal branches in the liver arterial trees for each cohort in FIG. 6A at 8 days post-LCMV-CI13 infection. **FIG. 6E** shows quantitation of mean vessel diameter determined in each branch individually at the indicated depths in ChAT^{WT} mice (black), T-ChAT^{KO} mice (white), T-ChAT^{KO} mice treated with minoxidil (light grey), and C57Bl6 mice treated with L-NAME (dark grey) examined at day 8 post-infection. Tukey box and whisker plot, line at median. Composite of measurements from n=4 day 8 *Chat*^{WT} and T-*Chat*^{KO}, and n=3 minoxidil and L-NAME treated mice. Two-way ANOVA was performed ($p < 0.0001$), followed by multiple comparisons between groups. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ **FIG. 6F** shows representative images of arterial trees acquired as in FIG. 6C from naïve ChAT^{WT} or T-ChAT^{KO} mice. Representative of 2 (naïve) mice. Scale bar is 200 μ M. **FIG. 6G** shows the mean vessel diameter in ChAT^{WT} (black) or T-ChAT^{KO} (white) livers (n=4) normalized to the average vessel diameter in livers of naïve mice (n=2) at day 8 post-infection at each branch depth. Values of greater than 100% (dashed line) indicate increased blood vessel diameter. Mean+SEM, average of n=2 naïve mice for each genotype used for comparison to values in n=4 day 8 infected samples in each genotype. Statistical significance determined by unpaired two-tailed *t*-test between day 8 and naïve of the same genotype.

[0060] FIG. 7A – FIG. 7K: **FIG. 7A** shows a schematic of experimental design in which ChAT^{WT} or T-ChAT^{KO} mice were infected with LCMV-CI13 and then gavaged daily with either water or minoxidil hydrochloride dissolved in water on days 6-12 post-infection. **FIG. 7B** shows serum viral titers of the mice in FIG. 7A at 30 days post-infection. **FIG. 7C, FIG. 7D, and FIG. 7E** show quantitation of numbers of virus-specific [D^b(GP33)+ or D^b(GP276)+] CD8+ T cells that had migrated to the liver (FIG. 7C), salivary gland (FIG. 7D), or lung (FIG. 7E) of the mice in FIG. 7A as determined by intravascular staining. **FIG. 7F, FIG. 7G, and FIG. 7H** show the fraction of tetramer+ T cells from treated mice in FIG. 7A and control mice with the same genotype in the liver after staining for PD-1, Tim-3, and LAG-3 and determining the amount expressing no

inhibitory receptors, one receptor, two receptors, or all three receptors. **FIG. 7I** shows a schematic of experimental design in which WT C57Bl6 mice were i.p.-injected with L-NAME on days 6-12 post-infection with LCMV-Cl13. **FIG. 7J** shows serum viral titers of the mice in FIG. 7I determined on the indicated days post-infection. **FIG. 7K** shows the fraction of tetramer⁺ cells from the spleen of a cohort of mice in FIG. 7I expressing no inhibitors or one, two, or three inhibitors, n=5 control, n=3 L-NAME treated *p<0.05, **p<0.01, ***p<0.001.

[0061] FIG. 8A and FIG. 8B show IL-21 induces *Chat* expression in vitro and in vivo. **FIG. 8A** shows ChAT expression by flow cytometry in P14 CD8 transgenic T cells in vitro after 5 days alone, in the presence of GP33 peptide, or in the presence of GP33 peptide plus the indicated cytokines. (related to FIG. 3A) *Chat*-GFP⁺ P14 (upper panels) or *Chat*-GFP⁻ P14 (bottom panel) CD8 T cells were cultured in vitro for 5 days either alone, in the presence of cognate GP33 peptide, or in the presence of GP33 peptide and indicated cytokines. Induction of *Chat*-GFP in P14 cells was evaluated after 5 days of culture by flow cytometry. Each panel represents P14 cells derived from an individual animal stimulated with indicated conditions. Representative of two experimental cohorts, n=5 for *Chat*-GFP⁺ P14, n=3 GFP⁻ P14 controls. **FIG. 8B** shows *il21r*^{+/+} (black) and *il21r*^{-/-} (white) mice expressing *Chat*-GFP were infected with LCMV-Cl13. Virus-specific CD8⁺ and CD4⁺ T cells were identified by tetramer staining, and the MFI for *Chat*-GFP within the GFP⁺ fraction was enumerated 8 days post-infection. Mean±S.E.M. Composite of 2-3 experimental cohorts, n=8-13. Statistical significance determined by unpaired two-tailed t-test *p<0.05, **p<0.01

[0062] FIG. 9A – FIG. 9M show loss of ChAT in T cells enhances expression of inhibitory receptors and impairs viral control, but does not influence proliferation. (related to FIG. 4) **FIG. 9A** shows numbers of D^b(GP33) (upper) and D^b(GP276) (lower) specific CD8⁺ T cells determined in the spleen of ChAT^{WT} and T-ChAT^{KO} mice infected with LCMV-Arm at the days indicated post-infection by tetramer staining. **FIG. 9B** shows representative flow plots of PD-1 expression by D^b(GP33) (upper) or D^b(GP276) (lower) splenic CD8⁺ T cells from the ChAT^{WT} (left) or T-ChAT^{KO} (right) mice in FIG. 9A at 30 days post-infection. **FIG. 9C** shows the fraction of D^b(GP33)⁺ (upper) and D^b(GP276)⁺ (lower) CD8⁺ T cells that expressed the proliferation marker Ki67 when ChAT^{WT} and T-ChAT^{KO} mice were infected with LCMV-Cl13. **FIG. 9D** shows representative flow plots of PD-1 and Ki67 expression by the D^b(GP33) (upper) and D^b(GP276) (lower) CD8⁺ splenocytes in FIG. 9C at 60 days post-infection. **FIG. 9E and FIG. 9F** show mean

fluorescence intensity (MFI) of PD-1, Tim-3 and LAG-3 on D^b(GP33)+ (FIG. 9E) and D^b(GP276)+ (FIG. 9F) CD8+ splenocytes from LCMV-CI13-infected ChAT^{WT} (black) and T-ChAT^{KO} (white) mice as determined by flow cytometry on the indicated days post-infection. **FIG. 9G and FIG. 9H** show representative flow plots demonstrating PD-1, Tim-3, and LAG-3 expression on the D^b(GP33)+ (FIG. 9G) or D^b(G276)+ (FIG. 9H) splenocytes in FIG. 9E and FIG. 9F at 60 days post-infection. **FIG. 9I and FIG. 9J** show IRF4 expression by D^b(GP33)+ (FIG. 9I) or D^b(GP276)+ (FIG. 9J) CD8+ T cells from ChAT^{WT} (black) and T-ChAT^{KO} (white) mice determined by flow cytometry at 30, 60, 120 days post-infection with LCMV-CI13. **FIG. 9K and FIG. 9L** show representative flow plots of the tetramer+ CD8+ T cells in FIG. 9I and FIG. 9J at 60 days post infection. **FIG. 9M** shows viral titers in the liver, lung, brain, and kidney tissues of ChAT^{WT} (black) and T-ChAT^{KO} (white) mice determined at the indicated days post-infection with LCMV-CI13.

[0063] FIG. 10A – FIG. 10D show loss of ChAT in T cells does not diminish anti-viral CD4+ T cell or B cell responses to LCMV-CI13. (related to Figure 4). **FIG. 10A** shows the number of LCMV-GP61-specific CD4+ T cells determined in ChAT^{WT} (black) and T-ChAT^{KO} (white) mice at 30 and 60 days post-infection with LCMV-CI13. Mean +S.E.M. Composite data of 2 experimental replicates, D30 n=8, D60 n=7 WT n=9 T-*Chat*^{KO}. **FIG. 10B** shows the number of splenic CD4+ T cells at indicated days post-infection in CHAT^{WT} (black) and T-CHAT^{KO} (white) mice capable of producing IFN γ following GP61 stimulation. Mean+S.E.M. Composite of at least 2 experimental cohorts, n=9-19. **FIG. 10C** shows the number of Fas+ GL-7+ GC B cells in the spleen of ChAT^{WT} (black) and T-ChAT^{KO} (white) mice at the indicated days post-infection. Mean+S.E.M. Composite data of 2-3 experimental replicates, WT n=9-12, T-*Chat*^{KO} n=10-12. **FIG. 10D** shows serum anti-LCMV IgG antibody titers determined by ELISA at the indicated days post-LCMV-CI13 infection in ChAT^{WT} (black) and T-ChAT^{KO} (white) mice. Each symbol represents an individual animal. Statistical significance determined by unpaired two-tailed t-test *p<0.05

[0064] FIG. 11A – FIG. 11I show inhibitory receptor expression in livers of ChAT^{WT} and T-ChAT^{KO} mice at 8 days post LCMV-CI13 infection and migration of T cells in IL-21R^{KO} mice. Mice were injected with α -CD8-FITC i.v. 3 minutes prior to sacrifice, ten livers were processed, washed and stained *ex vivo* for CD8, tetramer, and inhibitory receptors. (related to FIG. 5) **FIG. 11A and FIG. 11B** show mean fluorescence intensity (MFI) of PD-1, Tim-3, and LAG-3 expression by D^b(GP33)+ (FIG. 11A) or D^b(GP276)+ (FIG. 11B) liver infiltrating CD8+ lymphocytes on day 8 post-infection. **FIG. 11C and FIG. 11D** show the fraction of D^b(GP33)+ (FIG. 11C) or D^b(GP276)+ (FIG. 11D) liver infiltrating CD8+

lymphocytes from FIG. 11A and FIG. 11B expressing no inhibitory receptors, one receptor, two receptors, or all three receptors. **FIG. 11E and FIG. 11F** show the number of tetramer+ virus-specific cells in the liver circulation (FIG. 11E) and the number that migrated into liver tissue (FIG. 11F) for IL-21R^{WT} and IL-21R^{KO} mice 8 days post-infection with LCMV-Cl13 and stained intravascularly as in FIG. 11A. **FIG. 11G** shows numbers of P14 T cells in the spleens of the recipient mice in FIG. 5E 30 days post-infection. **FIG. 11H** shows the fraction of splenic P14 T cells of mice in FIG. 11G 30 days post-infection which express no inhibitory receptors, one receptor, two receptors, or all three receptors determined flow cytometry analysis of PD-1, Tim-3, and LAG-3 on splenic P14 T cells. **FIG. 11I** shows MFI determination of PD-1 and LAG-3 expression by transferred P14 T cells in the indicated recipients at 30 days post-infection.

[0065] FIG. 12A – FIG. 12F show that blood dilation is dynamic during the course of viral infection. (related to FIG. 6) **FIG. 12A** shows blood vessel diameter in formalin-fixed paraffin-embedded liver sections obtained from ChAT^{WT} or T-ChAT^{KO} mice infected with LCMV-Cl13 and analyzed on day 8 post-infection. **FIG. 12B** shows representative images of ChAT^{WT} and T-ChAT^{KO} livers stained with anti-CD31 (yellow) at 8 days post-infection. **FIG. 12C** shows blood vessel diameter measured in z-stacks obtained by 2-photon microscopy in ChAT^{WT} (gray) or T-ChAT^{KO} (white) liver at 8-9 days post-infection with LCMV-Cl13. **FIG. 12D** shows representative images of blood vessels in the livers in FIG. 12C. **FIG. 12E** shows mean vessel diameter in each branch individually at the indicated depths in naïve ChAT^{WT} and T-ChAT^{KO} mice. **FIG. 12F** shows quantitation of the number of terminal arterial branches in the livers of the naïve ChAT^{WT} or T-ChAT^{KO} mice in FIG. 12E.

[0066] FIG. 13 shows innate cell infiltration after vasodilator treatment in T-ChAT^{KO} mice with transplanted B16 melanoma.

[0067] FIG. 14 shows lymphocyte cell infiltration after vasodilator treatment in T-ChAT^{KO} mice with transplanted B16 melanoma.

[0068] FIG. 15A and FIG. 15B show loss of *Chat* expression and acetylcholine production in T cells from T-*Chat*^{KO} mice. **FIG. 15A** shows total CD4⁺ or CD8⁺ T cells isolated from pooled secondary lymphoid organs of *Chat*^{WT} (gray, n=8) or T-*Chat*^{KO} (white, n=9) mice 8 days post-LCMV Cl13 infection. Naïve CD44^{lo} GFP⁻ CD4⁺ and CD8⁺ cells were isolated by cell sorting from pooled secondary lymphoid organs of 5 *Chat*-GFP mice, and used as controls for *Chat* expression. Expression determined by the $\Delta\Delta$ CT method, using RSP9 as a housekeeping gene and normalized to either naïve

CD4⁺ (left) or CD8⁺ (right) cells. Results of one experimental cohort of pooled mice, run in technical triplicates. Mean $\pm$ S.E.M. **FIG. 15B** shows CD8⁺ CD44^{hi} effector cells sorted from pooled splenocytes of *Chat*^{WT} (gray) or T-*Chat*^{KO} (white) 8 days post-LCMV Armstrong infection restimulated in vitro for 15 minutes with anti-CD3 or unstimulated, and acetylcholine levels in the supernatant measured by mass spectrometry. Mean $\pm$ S.E.M. of 3-4 biological replicates from two experimental cohorts. N.D. – not detected. Statistical significance determined by unpaired two-tailed t-test * p <0.05, ** p <0.01

[0069] FIG. 16A and FIG. 16B show tumor growth and tumor mass of nAChR α 7 WT, nAChR α 7^{+/-}, and nAChR α 7^{-/-} mice with transplanted B16 melanoma.

[0070] FIG. 17A – FIG. 17D show *Chat* is induced in virus-specific T cells in an IL-21-dependant manner. **FIG. 17A and FIG. 17B** show that *Chat*-GFP⁺ and *Chat*-GFP⁻ animals were infected with LCMV-Arm and the expression of *Chat*-GFP in total CD4⁺ (FIG. 17A) or CD8⁺ (FIG. 17B) T cells 8 days post-infection was compared to expression in uninfected *Chat*-GFP⁺ cohorts. Mean $\pm$ S.E.M. Representative of 2-4 experimental cohorts, n=8-12 for *Chat*-GFP⁺, n=4 *Chat*-GFP⁻. **FIG. 17C and FIG. 17D** show that the fraction of virus-specific CD4⁺ (FIG. 17C) or CD8⁺ (FIG. 17D) T cells expressing *Chat*-GFP was determined 8, 20, and 30 days post-infection with LCMV-Arm or LCMV-Cl13 by evaluating tetramer staining and *Chat*-GFP expression by flow cytometry. Composite data of 2 (Arm) or 4 (Cl13) experiments. n=7-12 (Arm), n=10-27 (Cl13) animals per group per time point

[0071] FIG. 18A and FIG. 18B show that *Chat*-GFP was also induced in both CD4⁺ (FIG. 18A) and CD8⁺ (FIG. 18B) T cells following vesicular stomatitis virus (VSV) infection. *Chat*-GFP expression in CD4⁺ and CD8⁺ T cells from naïve (circles), or mice infected 8 days previously with 10⁶ pfu VSV Indiana (squares). Composite of 2 experimental cohorts, each symbol represents an individual mouse. Statistical significance determined by unpaired two-tailed t-test * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001.

[0072] FIG. 19A and FIG. 19B: FIG. 19A shows a representative flow plot of PD-1, Tim3, and LAG-3 expression in virus-specific CD8⁺ T cells 60 days post-infection in *Chat*^{WT} (black) or T-*Chat*^{KO} (gray) mice. **FIG. 19B** shows MFI 15 for PD-1, Tim3, and LAG-3 in virus-specific CD8⁺ T cells 60 days post-infection in *Chat*^{WT} (black) or T-*Chat*^{KO} (white) mice. Mean $\pm$ S.E.M., composite of 3 experimental cohorts n=13-15. Statistical significance for all samples determined by unpaired two-tailed *t*-test * p <0.05, ** p <0.01, *** p <0.001

[0073] FIG. 20A – FIG. 20D show IL-21-driven *Chat* expression in T cells facilitates migration into infected tissues. **FIG. 20A** shows day 8 LCMV-Cl13 infected *Il21r*^{+/+}

(black) or *Il21r^{-/-}* (grey) CD8-FITC i.v. 3 minutes prior to sacrifice. Livers were then processed, washed, and stained for CD8, tetramer, and inhibitory receptors. FITC⁺ liver-infiltrating CD8⁺ T cells were enumerated. Composite of 3 experimental cohorts, n=10-11 **FIG. 20B and FIG. 20C** show the number of virus-specific CD8 T cells in the tissue of *Chat^{WT}* (black) and T-*Chat^{KO}* (white) mice was determined in liver (FIG. 20B) and salivary gland (FIG. 20C) by intravascular staining as in FIG. 20A. Mean+S.E.M.

Composite of 5 experimental cohorts for liver, 2 experimental cohorts for salivary gland, n=12-22. **FIG. 20D** shows in vivo cytolytic activity was determined in the spleen and liver of *Chat^{WT}* or T-*Chat^{KO}* mice 8 days post-LCMV CI13 infection. Mean+S.E.M. Composite of 3-4 experimental cohorts, n=15 (GP276 and NP396); n=23 (GP33).

[0074] FIG. 21A – FIG. 21C: **FIG. 21A** shows *Chat^{WT}* or T-*Chat^{KO}* animals were infected with LCMV-CI13 and then gavaged with either water (control) or minoxidil hydrochloride dissolved in water daily on days 6-12 post-infection. Serum viral titer was determined 30 days post-infection in *Chat^{WT}* control (black), *Chat^{WT}* minoxidil-treated (dark grey), T-*Chat^{KO}* control (white) or T-*Chat^{KO}* minoxidil-treated (light grey) mice. Composite of 3-4 experimental cohorts, n=10-18. **FIG. 21B** shows serum viral titers of C57Bl/6 mice injected with either PBS (black) or L-NAME (grey) on days 6-12 post-LCMV-CI-13 infection. Composite of 3 experimental cohorts, n=10-15. **FIG. 21 C** shows *Il21r^{+/+}* or *Il21r^{-/-}* animals infected with LCMV-CI13 and then gavaged with either water or minoxidil daily on days 6-12 post-infection. Serum viral titer was determined 30 days post-infection in *Il21r^{+/+}* control (black), *Il21r^{+/+}* minoxidil-treated (cluster of grey squares second from left), *Il21r^{-/-}* control (white) or *Il21r^{-/-}* minoxidil-treated (grey circles) mice. Each symbol represents individual mice, n=4-7. Statistical significance for all samples determined by unpaired two-tailed *t*-test **p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001

[0075] FIG. 22 shows *Chat* expression in T cells is not necessary for control of acute viral infection. *Chat^{WT}* (black) and T-*Chat^{KO}* (white) animals were infected with LCMVArm. The number of D^b(GP33) (left) and D^b(GP276) (right) specific CD8⁺ T cells was elucidated in the spleen at indicated days post-infection by tetramer staining. Mean+/-S.E.M. Composite or representative of 2-3 experimental cohorts, WT D8 n=10, D12 and D120 n=9, D30 n=6. T-*Chat*-KO D8 n=10, D12 and D120 n=8, D30 n=6. No statistically significant differences were observed by unpaired two-tailed *t*-test.

[0076] FIG. 23A – FIG. 23G show *Il21r* and *Chat* expression does not alter the number of virus-specific cells in circulation. **FIG. 23A** shows day 8 LCMV-CI13 infected *Il21r^{WT}* (dark grey) or *Il21r^{KO}* (light grey) animals injected with α-CD8-FITC i.v. 3

minutes prior to sacrifice. Livers were then processed, washed, and stained for α -CD8-eFlour450 and either D^b(GP33)-APC or D^b(GP276)-APC tetramers. FITC⁺ circulating CD8⁺ T cells were enumerated. Composite of three experimental cohorts, n=10-11 **FIG. 23B and FIG. 23C** show the number of virus-specific CD8 T cells in the circulation of *Chat*^{WT} (black) and T*Chat*^{KO} (white) mice determined 8 days post-LCMV CI-13 in liver (FIG. 23B) and salivary gland (FIG. 23C) by intravascular staining as in FIG. 23A. Mean+S.E.M. Composite of five experimental cohorts for liver, two experimental cohorts for salivary gland, n=12-22. **FIG. 23D** shows the number of non-circulating and circulating virus-specific CD4⁺ T cells in the liver determined by intravascular staining with α -CD45.2-FITC as in FIG. 23A. Mean+/-S.E.M. Composite of two experimental cohorts, n=10-12. **FIGS. 23E-G** show *Chat*^{WT} P14 or *Chat*^{KO} P14 T cells transferred into either *Chat*^{WT} or T-*Chat*^{KO} recipient mice, which were subsequently infected with LCMV-CI13. Vascular cells in different tissues were marked as in FIG 23A 30 days post-infection. **FIG. 23E and FIG 23F** show the total number of P14 cells in recipient spleens (FIG. 23E; from left to right: WT→WT, KO→KO, WT→KO and KO→WT) or organs (FIG. 23F; from left to right in each organ: WT→WT, KO→KO, WT→KO and KO→WT) determined 30 days post-transfer. **FIG. 23G** shows the percentage of non-circulating D^b(GP33)⁺ T cells that are either endogenous (left hand column for each organ in all plots) or P14 (right hand column for each organ in all plots) in origin determined 30 days post infection. Composite of 2 experimental cohorts for controls and 4 experimental cohorts, n=5-10 control animals, n=12-17 experimental animals. Statistical significance determined by unpaired two-tailed t-test.

DETAILED DESCRIPTION OF THE INVENTION

[0077] The practice of the present invention may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such conventional techniques include polymer array synthesis, hybridization, ligation, phage display, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the example herein below. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV), *Using Antibodies: A Laboratory Manual*, *Cells: A*

Laboratory Manual, PCR Primer: A Laboratory Manual, and Molecular Cloning: A Laboratory Manual (all from Cold Spring Harbor Laboratory Press), Stryer, L. (1995) *Biochemistry* (4th Ed.) Freeman, New York, Gait, "Oligonucleotide Synthesis: A Practical Approach" 1984, IRL Press, London, Nelson and Cox (2000), *Lehninger, Principles of Biochemistry* 3rd Ed., W. H. Freeman Pub., New York, N.Y. and Berg et al. (2002) *Biochemistry*, 5th Ed., W. H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entirety by reference for all purposes.

[0078] Note that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polymerase" refers to one agent or mixtures of such agents, and reference to "the method" includes reference to equivalent steps and methods known to those skilled in the art, and so forth.

[0079] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing devices, compositions, formulations and methodologies which are described in the publication and which might be used in connection with the presently described invention.

[0080] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

[0081] In the following description, numerous specific details are set forth to provide a more thorough understanding of the present invention. However, it will be apparent to one of skill in the art that the present invention may be practiced without one or more of these specific details. In other instances, well-known features and procedures well known to those skilled in the art have not been described in order to avoid obscuring the invention.

[0082] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others.

“Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the composition or method.

“Consisting of” shall mean excluding more than trace elements of other ingredients for claimed compositions and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention. Accordingly, it is intended that the methods and compositions can include additional steps and components (comprising) or alternatively including steps and compositions of no significance (consisting essentially of) or alternatively, intending only the stated method steps or compositions (consisting of).

[0083] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term “about”. The term “about” also includes the exact value “X” in addition to minor increments of “X” such as “X + 0.1” or “X – 0.1.” It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0084] A “composition” may include any substance comprising an agent or compound and is also intended to encompass any combination of an agent or compound and other substances, including a carrier, e.g., compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Carriers also include pharmaceutical excipients and additives proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. Carbohydrate excipients are also intended within the scope of this invention, examples of which include but are not limited to monosaccharides such as

fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol) and myoinositol.

[0085] The term pharmaceutically acceptable carrier (or medium), which may be used interchangeably with the term biologically compatible carrier or medium, refers to reagents, cells, compounds, materials, compositions, and/or dosage forms that are not only compatible with the cells and other agents to be administered therapeutically, but also are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other complication commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable carriers suitable for use in the present invention include liquids, semi-solid (e.g., gels) and solid materials (e.g., cell scaffolds and matrices, tubes sheets and other such materials as known in the art and described in greater detail herein). These semi-solid and solid materials may be designed to resist degradation within the body (non-biodegradable) or they may be designed to degrade within the body (biodegradable, bioerodable). A biodegradable material may further be bioresorbable or bioabsorbable, i.e., it may be dissolved and absorbed into bodily fluids (water-soluble implants are one example), or degraded and ultimately eliminated from the body, either by conversion into other materials or breakdown and elimination through natural pathways.

[0086] As used herein, the term “patient” or “subject” intends an animal, a mammal or yet further a human patient. For the purpose of illustration only, a mammal includes but is not limited to a human, a simian, a murine, a bovine, an equine, a porcine or an ovine.

[0087] As used herein, the term “choline acetyltransferase” or “ChAT” refers to the enzyme which catalyzes the rate-limiting step of ACh production.

[0088] As used herein the term “vasodilator” refers to any drugs associated with modulating blood flow or lowering blood pressure. In some embodiments, vasodilator refers to arterial dilators, mainly affecting the arteries. In some embodiments, vasodilator refers to venous dilators, mainly affecting the veins. In some embodiments, vasodilator refers to mixed dilators, affecting veins and arteries.

[0089] As used herein, the term “biological activity of the vasodilator” or “vasodilator activity” refers to any biological activity associated with the vasodilator. In some embodiments, the biological activity of the vasodilator refers to increasing vasodilation. In some embodiments, the biological activity of the vasodilator refers to ACh production. In further embodiments, the biological activity of the vasodilator refers to mediating protection from endotoxemia observed with vagus nerve stimulation. In yet further embodiments, the biological activity of the vasodilator refers to migration of cells into tissues. In yet further embodiments, the biological activity of the vasodilator refers to migration of inflammatory T cells into tumors. In further embodiments, the biological activity of the vasodilator refers to migration of suppressive T cells into inflamed tissue. In further embodiments, the biological activity of the vasodilator refers to increasing clearance of infection. Activation threshold can be measured by increased expression of ChAT. In further embodiments, the biological activity of the vasodilator includes the activation of T cells.

[0090] As used herein, the term “treating” refers to administering a pharmaceutical composition for the purpose of improving the condition of a patient by reducing, alleviating, reversing, or preventing at least one adverse effect or symptom of a disease or disorder.

[0091] As used herein, the term “preventing” refers to identifying a subject (i.e., a patient) having an increased susceptibility to a disease but not yet exhibiting symptoms of the disease, and administering a therapy according to the principles of this disclosure. The preventive therapy is designed to reduce the likelihood that the susceptible subject will later become symptomatic or that the disease will be delayed in onset or progress more slowly than it would in the absence of the preventive therapy. A subject may be identified as having an increased likelihood of developing the disease by any appropriate method including, for example, by identifying a family history of the disease or other degenerative brain disorder, or having one or more diagnostic markers indicative of disease or susceptibility to disease.

[0092] As used herein, the term “sample” or “test sample” refers to any liquid or solid material containing nucleic acids. In suitable embodiments, a test sample is obtained from a biological source (i.e., a “biological sample”), such as cells in culture or a tissue sample from an animal, most preferably, a human.

[0093] A “biological equivalent” of a protein or nucleic acid refers to a protein or nucleic acid that is substantially identical to the protein or nucleic acid by amino acid or nucleic acid sequence or that has an equivalent biological activity.

[0094] As used herein, the term “effective amount” refers to a quantity of compound (e.g., a vasodilator) delivered with sufficient frequency to provide a medical benefit to the patient. In one embodiment, an effective amount of a vasodilator is an amount sufficient to treat or ameliorate a symptom of a disease.

[0095] A population of cells intends a collection of more than one cell that is identical (clonal) or non-identical in phenotype and/or genotype.

[0096] As used herein, an “antibody” includes whole antibodies and any antigen binding fragment or a single chain thereof. Thus the term “antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein. In general, the term “antibody” includes any polypeptide that includes at least one constant domain, including, but not limited to, CH1, CH2, CH3 and CL. Antibodies that find use in the present invention can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics.

[0097] The antibodies can be polyclonal or monoclonal and can be isolated from any suitable biological source, e.g., murine, rat, sheep and canine.

[0098] A monoclonal antibody is an antibody produced by a single clone of cells or a hybridoma, and therefore is a single pure homogeneous type of antibody.

[0099] A hybridoma is a cell that is produced in the laboratory from the fusion of an antibody-producing lymphocyte and a non-antibody producing cancer cell, usually a myeloma or lymphoma. A hybridoma proliferates and produces a continuous supply of a specific monoclonal antibody.

[00100] The term “human antibody” as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*).

However, the term “human antibody” as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Thus, as used herein, the term “human antibody” refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C_L, C_H domains (e.g., C_{H1}, C_{H2}, C_{H3}), hinge, (VL, VH)) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

[00101] The term “recombinant human antibody”, as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the

recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*. Methods to making these antibodies are described herein.

[00102] “Isotype” as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. It should be understood that therapeutic antibodies can also comprise hybrids of isotypes and/or subclasses.

[00103] The terms “polyclonal antibody” or “polyclonal antibody composition” as used herein refer to a preparation of antibodies that are derived from different B-cell lines. They are a mixture of immunoglobulin molecules secreted against a specific antigen, each recognizing a different epitope.

[00104] The terms “monoclonal antibody” or “monoclonal antibody composition” as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[00105] The terms “Systemically” or “systemic delivery” as used herein refer to delivering a vasodilator such that the entire body is affected. In some embodiments “systemically” refers to oral or intravenous delivery.

[00106] The terms “Locally” or “local delivery” as used herein refer to delivering a vasodilator such that it does not affect the entire body. In other embodiments, “locally” refers to injection or application at the site of the tumor. In other embodiments, “locally” refers to injection or application at the site of inflammation.

[00107] The term “tumor infiltrating lymphocytes” as used herein refers to lymphocytes which have left the blood stream and migrated towards a tumor.

[00108] Although the present invention is described primarily with reference to specific embodiments, it is also envisioned that other embodiments will become apparent to those skilled in the art upon reading the present disclosure, and it is intended that such embodiments be contained within the present inventive methods.

Overview of the invention

[00109] The present disclosure provides methods and compositions for modulating choline acetyltransferase (which is also interchangeably referred to herein as “ChAT”) in T cells. Such modulation may include modulation of ChAT expression in T cells and/or modulation of ChAT activity in T cells.

[00110] In some examples, the present disclosure describes methods and compositions for increasing or decreasing ChAT expression in T cells. In some examples, the present disclosure describes compositions comprising T cells that have been engineered to increase expression of ChAT as well as methods for such increased expression. In some examples, methods and compositions described herein include engineering a virus to mediate induction of ChAT.

[00111] In other examples, the present disclosure describes methods and composition for increasing or decreasing ChAT activity in T cells.

[00112] In still further examples, the present disclosure encompasses methods of treating cancer and viral infection by measuring the level of ChAT in T cells in a patient, assessing whether the patient has low levels of ChAT, and if low levels are identified, administering to the patient a vasodilator to overcome the effects of low ChAT expression in T cells.

1. Methods of modulating ChAT expression in T cells

[00113] In one aspect, the present invention provides methods and compositions for modulating ChAT expression in T cells. As will be appreciated, modulating ChAT expression in T cells refers to either increasing or decreasing expression of ChAT.

A. Increasing ChAT expression in T cells

1. Compositions increasing ChAT expression in T cells

[00114] In some embodiments, modulating ChAT expression in T cells comprises administering a composition that increases ChAT expression in T cells. In some embodiments, ChAT expression in T cells is increased directly. In further embodiments, ChAT expression in T cells is increased directly by administering a therapeutically effective pharmaceutical composition comprising ChAT. In other embodiments, ChAT expression in T cells may be increased indirectly. In further embodiments, ChAT expression in T cells may be increased indirectly by administering a therapeutically effective pharmaceutical composition modulating the IL-21 pathway. In some embodiments, ChAT expression in T cells may be increased indirectly by administering a therapeutically effective pharmaceutical composition comprising IL-21.

[00115] In further aspects, compositions of the invention may include additives and pharmaceutically acceptable carriers. As used herein, "pharmaceutically acceptable carrier" includes any material, which when combined with the composition retains the composition's activity and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well-known conventional methods.

2. Administering T cells engineered to increase ChAT expression in T cells

[00116] In other embodiments, modulating ChAT expression in T cells comprises engineering T cells to increase expression of ChAT. Embodiments of the present invention encompass genetic editing through nucleotide insertion (DNA or RNA) into a population of T cells for promotion of expression of one or more proteins. There are several gene-editing technologies known in the art that may be used to genetically modify a population of T cells which are suitable for use in accordance with the present invention. See *Cox et al. Nature Medicine, 2015, Vol. 21, No. 2*. In some embodiments, methods for enabling site-specific genomic editing of T cells include, but are not limited to Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-associated nucleases, transcription activator-like nucleases (TALEN), and zinc finger nuclease (ZFN) systems.

[00117] Examples of systems, methods, and compositions for altering a target gene sequence by a CRISPR method, and which may be used in accordance with the embodiments of the present invention are described in U.S. patent Nos. 8,697,359; 8,993,233; 8,795,965; 8,771,945; 8,889,356; 8,865,406; 8,999,641; 8,945,839; 8,932,814; 8,871,445; 8,906,616; and 8,895,308.

[00118] "Transcription Activator-Like Effector" proteins are naturally occurring proteins from the plant pathogenic bacteria genus *Xanthomonas*, and contain DNA-binding

domains composed of a series of 33-35-amino-acid repeat domains that each recognize a single base pair. TALE specificity is determined by two hypervariable amino acids that are known as the repeat-variable di-residues (RVDs). Modular TALE repeats are linked together to recognize contiguous DNA sequences. A specific RVD in the DNA-binding domain recognizes a base in the target locus, providing a structural feature to assemble predictable DNA-binding domains. The DNA binding domains of TALE are fused to the catalytic domain of a type IIS Fok1 endonuclease to make a targetable TALE nuclease. To induce site-specific mutation, two individual TALEN arms, separated by a 14-20 base pair spacer region, bring Fok1 monomers in close proximity to dimerize and produce a targeted double-strand break. TALE and TALEN methods suitable for use in the present invention are described in U.S. Patent Application Publication Nos. US2011/0201118 A1; US2013/0117869 A1; US2013/0315884 A1; US2015/0203871 A1; and US2016/0120906.

[00119] Zinc finger gene-editing or zinc finger nuclease methods may also be used for gene editing. The DNA-binding domains of individual ZFNs typically contain between three and six individual zinc finger repeats and can each recognize between 9 and 18 base pairs. If the zinc finger domains are specific for their intended target site then even a pair of 3-finger ZFNs that recognize a total of 18 base pairs can, in theory, target a single locus in a mammalian genome. Examples of systems, methods and compositions for altering the expression of a target gene sequence by a zinc finger method, which may be used in accordance with embodiments of the present invention, are described in U.S. Patent Nos. 6,534,261, 6,607,882, 6,746,838, 6,794,136, 6,824,978, 6,866,997, 6,933,113, 6,979,539, 7,013,219, 7,030,215, 7,220,719, 7,241,573, 7,241,574, 7,585,849, 7,595,376, 6,903,185, and 6,479,626. Other examples of systems, methods and compositions for altering the expression of a target gene sequence by a zinc finger method, which may be used with the present invention, are described in Beane, *et. al*, *Mol. Therapy*, **2015**, 23 1380-1390.

[00120] T cells can be taken from the subject to be treated or from a donor subject. In some embodiments, the engineered T cells are autologous. In some embodiments, the engineered T cells are allogenic. In some embodiments, the engineered T cells are antigen-specific.

3. Administering a virus engineered to increase ChAT expression in T cells

[00121] In other embodiments, modulating ChAT expression in T cells comprises administering a virus that is engineered to increase ChAT expression in T cells. In some embodiments, the virus is engineered to directly increase ChAT expression in T cells. In other embodiments, the virus is engineered to indirectly increase ChAT expression in T cells. In further embodiments, the engineered virus indirectly increases ChAT expression in T cells by inducing the IL-21 pathway. Methods to engineer viruses for increasing ChAT expression in T cells include those performed by a person skilled in the art in accordance with any of the methods described herein. Examples of viruses that may be used include but are not limited to adenoviruses, lentiviruses, retrovirus, herpes simplex viruses, and adeno-associated viruses.

B. Decreasing ChAT expression in T cells

[00122] In some embodiments, modulating ChAT expression in T cells comprises administering a composition that decreases ChAT expression in T cells. In some embodiments, ChAT expression in T cells is decreased directly. In further embodiments, ChAT expression in T cells is decreased directly by administering a therapeutically effective pharmaceutical composition comprising an antibody that binds to ChAT. In other embodiments, ChAT expression in T cells is decreased by administering a therapeutically effective pharmaceutical composition comprising siRNA to silence the expression of ChAT. In other embodiments, ChAT expression in T cells may be decreased indirectly. In further embodiments, ChAT expression in T cells may be decreased indirectly by administering a therapeutically effective pharmaceutical composition modulating the TGF- β pathway. In some embodiments, ChAT expression in T cells may be decreased indirectly by administering a therapeutically effective pharmaceutical composition comprising TGF- β .

II. Methods of modulating ChAT activity in T cells

[00123] In another aspect, the present invention provides methods and compositions for modulating ChAT activity in T cells. As will be appreciated, modulating ChAT activity in T cells refers to either increasing or decreasing activity of ChAT.

A. Increasing ChAT activity in T cells

1. Compositions that increase ChAT activity in T cells

[00124] In some embodiments, modulating ChAT activity in T cells comprises administering a composition that increases ChAT activity in T cells. In some embodiments, ChAT activity in T cells is increased directly. In further embodiments, ChAT activity in T cells is increased by administering a therapeutically effective pharmaceutical composition comprising ChAT with modifications to enhance activity. Potential methods to increase activity include without limitation enhancing affinity, half-life, efficiency, and the like.

[00125] In further aspects, compositions of the invention may include additives and pharmaceutically acceptable carriers. As used herein, "pharmaceutically acceptable carrier" includes any material, which when combined with the conjugate retains the conjugates' activity and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well-known conventional methods.

2. Administering T cells engineered to increase ChAT activity in T cells

[00126] In other embodiments, increasing ChAT activity in T cells comprises engineering T cells to increase activity of ChAT. In some embodiments, methods for engineering T cells include those stated above. In some embodiments, the engineered T cells are autologous. In some embodiments, the engineered T cells are allogenic. In some embodiments, the engineered T cells are antigen-specific.

3. Administering a virus engineered to increase ChAT activity in T cells

[00127] In other embodiments, modulating ChAT activity in T cells comprises administering a virus that is engineered to increase ChAT activity in T cells. In some embodiments, the virus is engineered to directly increase ChAT activity in T cells. In other embodiments, the virus is engineered to indirectly increase ChAT activity in T cells. Viral engineering can be performed using any methods known to a person skilled in the art.

B. Decreasing ChAT activity in T cells

1. Compositions that decrease ChAT activity in T cells

[00128] In some embodiments, modulating ChAT activity in T cells comprises administering a composition that decreases ChAT activity in T cells. In some embodiments, ChAT activity in T cells is decreased directly. In further embodiments, ChAT activity in T cells is decreased by administering a therapeutically effective pharmaceutical composition comprising an antibody that binds to ChAT. In some embodiments, ChAT activity in T cells is decreased by administering a therapeutically effective pharmaceutical composition comprising ChAT with modifications. Potential methods to decrease activity comprise modifications that lower affinity, half-life, efficiency, etc.

[00129] In further aspects, compositions of the invention may include additives and pharmaceutically acceptable carriers. As used herein, "pharmaceutically acceptable carrier" includes any material, which when combined with the conjugate retains the conjugates' activity and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well-known conventional methods.

2. Administering T cells engineered to decrease ChAT activity in T cells

[00130] In other embodiments, decreasing ChAT activity in T cells comprises engineering T cells to decrease activity of ChAT. In some embodiments, methods for engineering T cells include those stated above. In some embodiments, the engineered T cells are autologous. In some embodiments, the engineered T cells are allogenic. In some embodiments, the engineered T cells are antigen-specific.

3. Administering a virus engineered to decrease ChAT activity in T cells

[00131] In other embodiments, modulating ChAT activity in T cells comprises administering a virus that is engineered to decrease ChAT activity in T cells. In some embodiments, the virus is engineered to directly decrease ChAT activity in T cells. In other embodiments, the virus is engineered to indirectly decrease ChAT activity in T cells. Viral engineering can be performed using any methods known to a person skilled in the art.

III. Treating cancer or viral infection by modulating ChAT expression in T cells

[00132] In another aspect, the present invention provides methods and compositions for treating cancer by modulating ChAT expression in T cells. In some embodiments, ChAT expression in T cells leads to vasodilation. In some embodiments, ChAT catalyzes the rate-limiting step of acetylcholine (ACh) production. ACh is produced by the acetylation of choline, with acetyl-CoA as the donor in the presence of CHAT. In some embodiments, ACh production induces vasodilation. In some embodiments, an increase in ChAT expression in T cells results in increased vasodilation. In some embodiments, increasing expression of ChAT increases the ability of a therapeutic agent to reach a target tissue. In some embodiments, the disease or disorder to be treated is cancer. In other embodiments, the disease or disorder to be treated is viral infection.

A. Cancer

1. Modulating ChAT expression in T cells to treat cancer

[00133] In some embodiments, increasing expression of ChAT in T cells increases the ability of the body to fight cancer. In some embodiments, increasing expression of ChAT increases the ability of a therapeutic agent to reach a tumor. In other embodiments, increasing expression of ChAT in T cells increases leucocytes in a tumor. In other embodiments, increasing ChAT expression in T cells increases lymphocytes in a tumor. In yet other embodiments, increasing ChAT expression in T cells increases T cells in a tumor. In other embodiments, increasing ChAT expression in T cells increases CD4+ T cells in a tumor. In other embodiments, increasing ChAT expression in T cells increases CD4+CXCR5+PD-1+ T cells in a tumor. In other embodiments, increasing ChAT expression in T cells increases CD8+ T cells in a tumor. In some embodiments, increasing ChAT expression in T cells increases B cells in a tumor. In other embodiments, increasing ChAT expression in T cells increases CD11b+ cells in a tumor. In other embodiments, increasing ChAT expression in T cells increases dendritic cells in a tumor. In other embodiments, increasing ChAT expression in T cells increases neutrophil cells in a tumor. In other embodiments, increasing expression of ChAT increases the number of tumor infiltrating lymphocyte cells present in a tumor.

[00134] In some embodiments, the tumor infiltrating lymphocyte cells that increase in the tumor due to increased ChAT expression in T cells slow tumor growth. In other embodiments the tumor infiltrating lymphocyte cells that increase in the tumor due to increased ChAT expression in T cells reduce tumor size. In other embodiments, the tumor infiltrating lymphocyte cells kill cancer cells.

2. Modulating ChAT expression in T cells in combination with other therapeutic agents for cancer

[00135] In some embodiments, methods or compositions that modulate ChAT expression in T cells can be combined with other therapeutic agents for the treatment of cancer.

[00136] In some embodiments, the composition for treating cancer may be combined with one or more anti-cancer therapeutic agents to enhance an anti-cancer response. In some embodiments at least one of the anti-cancer therapeutics is an immune

checkpoint inhibitor. In further embodiments, the at least one checkpoint inhibitor is a member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a Tim-3 inhibitor, and a Lag-3 inhibitor. In some embodiments, these checkpoint inhibitors serve to increase tumor cell killing and clearance.

[00137] Exemplary therapeutic agents that may be used as part of a combination therapy in treating cancer, include, for example, radiation, mitomycin, tretinoin, ribomustin, gemcitabine, vincristine, etoposide, cladribine, mitobronitol, methotrexate, doxorubicin, carboquone, pentostatin, nitracrine, zinostatin, cetorelix, letrozole, raltitrexed, daunorubicin, fadrozole, fotemustine, thymalfasin, sobuzoxane, nedaplatin, cytarabine, bicalutamide, vinorelbine, vesnarinone, aminoglutethimide, amsacrine, proglumide, elliptinium acetate, ketanserin, doxifluridine, etretinate, isotretinoin, streptozocin, nimustine, vindesine, flutamide, drogenil, butocin, carmofur, razoxane, sizofilan, carboplatin, mitolactol, tegafur, ifosfamide, prednimustine, picibanil, levamisole, teniposide, improsulfan, enocitabine, lisuride, oxymetholone, tamoxifen, progesterone, mepitiostane, epitio stanol, formestane, interferon-alpha, interferon-2 alpha, interferon-beta, interferon-gamma, colony stimulating factor-1, colony stimulating factor-2, denileukin diftitox, interleukin-2, luteinizing hormone releasing factor and variations of the aforementioned agents that may exhibit differential binding to its cognate receptor, and increased or decreased serum half-life.

[00138] An additional class of agents that may be used as part of a combination therapy in treating cancer is immune checkpoint inhibitors. Exemplary immune checkpoint inhibitors include agents that inhibit one or more of (i) cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), (ii) programmed cell death protein 1 (PD1), (iii) PDL1, (iv) LAG3, (v) B7-H3, (vi) B7-H4, and (vii) TIM3, such as Ipilimumab, Nivolumab, Pembrolizumab, Avelumab, Durvalumab, and Atezolizumab.

[00139] Yet other agents that may be used as part of a combination therapy in treating cancer are monoclonal antibody agents that target non-checkpoint targets (e.g., herceptin) and non-cytotoxic agents (e.g., tyrosine-kinase inhibitors).

[00140] Yet other categories of anti-cancer agents include, for example: (i) an inhibitor selected from an ALK Inhibitor, an ATR Inhibitor, an A2A Antagonist, a Base Excision Repair Inhibitor, a Bcr-Abl Tyrosine Kinase Inhibitor, a Bruton's Tyrosine Kinase Inhibitor, a CDC7 Inhibitor, a CHK1 Inhibitor, a Cyclin-Dependent Kinase Inhibitor, a DNA-PK Inhibitor, an Inhibitor of both DNA-PK and mTOR, a DNMT1 Inhibitor, a DNMT1 Inhibitor plus 2-chloro-deoxyadenosine, an HDAC Inhibitor, a

Hedgehog Signaling Pathway Inhibitor, an IDO Inhibitor, a JAK Inhibitor, a mTOR Inhibitor, a MEK Inhibitor, a MELK Inhibitor, a MTH1 Inhibitor, a PARP Inhibitor, a Phosphoinositide 3-Kinase Inhibitor, an Inhibitor of both PARP1 and DHODH, a Proteasome Inhibitor, a Topoisomerase-II Inhibitor, a Tyrosine Kinase Inhibitor, a VEGFR Inhibitor, and a WEE1 Inhibitor; (ii) an agonist of OX40, CD137, CD40, GITR, CD27, HVEM, TNFRSF25, or ICOS; and (iii) a cytokine selected from IL-12, IL-15, GM-CSF, and G-CSF.

[00141] Antibodies of the invention can also be used as an adjunct to surgical removal of cancer from the primary lesion.

[00142] In some embodiments, the composition modulating ChAT expression in T cells is conjugated to another anti-cancer therapeutic agent. Conjugation can occur through any method known to a person skilled in the art. In some embodiments, conjugation of the composition modulating ChAT expression in T cells may be to an antibody. In some embodiments, the composition modulating ChAT expression in T cells may be conjugated to a small molecule. In some embodiments, the composition modulating ChAT expression in T cells may be a component of an antibody drug conjugate. In other embodiments the composition modulating ChAT expression in T cells may be conjugated to a peptide. In some embodiments, the composition modulating ChAT expression in T cells may be a component of a peptide drug conjugate. In some embodiments, the composition modulating ChAT expression in T cells may be conjugated to an oligonucleotide.

3. Cancer types to be treated

[00143] The present disclosure provides methods of treating cancer. In some embodiments, the cancer to be treated is responsive to existing immune-modulating antibodies targeting other immune checkpoints, such as CTLA-4, PD-1 or PD-L1. In some embodiments, the cancer to be treated is non-responsive to existing immune-modulating antibodies targeting other immune checkpoints, such as CTLA-4, PD-1 or PD-L1.

[00144] In some embodiments, the cancer to be treated is melanoma.

[00145] In some embodiments, the cancer is a solid tumor, such as gastric cancer, colorectal cancer, hepatocellular carcinoma, or esophageal squamous cell carcinoma.

In some embodiments, the cancer is B-cell chronic lymphocytic leukemia, Hodgkin's lymphoma, B-cell non-Hodgkin's lymphoma or T-cell non-Hodgkin's lymphomas.

[00146] In some other embodiments, the cancer is brain cancer, bladder cancer, breast cancer, cervical cancer, endometrial cancer, esophageal cancer, leukemia, lung cancer, liver cancer, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, renal cancer, testicular cancer, or uterine cancer. In yet other embodiments, the cancer is a vascularized tumor, squamous cell carcinoma, adenocarcinoma, small cell carcinoma, neuroblastoma, sarcoma (e.g., an angiosarcoma or chondrosarcoma), larynx cancer, parotid cancer, biliary tract cancer, thyroid cancer, acral lentiginous melanoma, actinic keratoses, acute lymphocytic leukemia, acute myeloid leukemia, adenoid cystic carcinoma, adenomas, adenosarcoma, adenosquamous carcinoma, anal canal cancer, anal cancer, anorectum cancer, astrocytic tumor, Bartholin gland carcinoma, basal cell carcinoma, biliary cancer, bone cancer, bone marrow cancer, bronchial cancer, bronchial gland carcinoma, carcinoid, cholangiocarcinoma, chondrosarcoma, choroid plexus papilloma/carcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, clear cell carcinoma, connective tissue cancer, cystadenoma, digestive system cancer, duodenum cancer, endocrine system cancer, endodermal sinus tumor, endometrial hyperplasia, endometrial stromal sarcoma, endometrioid adenocarcinoma, endothelial cell cancer, ependymal cancer, epithelial cell cancer, Ewing's sarcoma, eye and orbit cancer, female genital cancer, focal nodular hyperplasia, gallbladder cancer, gastric antrum cancer, gastric fundus cancer, gastrinoma, glioblastoma, glucagonoma, heart cancer, hemangioblastomas, hemangioendothelioma, hemangiomas, hepatic adenoma, hepatic adenomatosis, hepatobiliary cancer, hepatocellular carcinoma, Hodgkin's disease, ileum cancer, insulinoma, intraepithelial neoplasia, interepithelial squamous cell neoplasia, intrahepatic bile duct cancer, invasive squamous cell carcinoma, jejunum cancer, joint cancer, Kaposi's sarcoma, pelvic cancer, large cell carcinoma, large intestine cancer, leiomyosarcoma, lentigo maligna melanomas, lymphoma, male genital cancer, malignant melanoma, malignant mesothelial tumors, medulloblastoma, medulloepithelioma, meningeal cancer, mesothelial cancer, metastatic carcinoma, mouth cancer, mucoepidermoid carcinoma, multiple myeloma, muscle cancer, nasal tract cancer, nervous system cancer, neuroepithelial adenocarcinoma nodular melanoma, non-epithelial skin cancer, oat cell carcinoma, oligodendroglial cancer, oral cavity cancer, osteosarcoma, papillary serous adenocarcinoma, penile cancer, pharynx

cancer, pituitary tumors, plasmacytoma, pseudosarcoma, pulmonary blastoma, rectal cancer, renal cell carcinoma, respiratory system cancer, retinoblastoma, rhabdomyosarcoma, sarcoma, serous carcinoma, sinus cancer, skin cancer, small cell carcinoma, small intestine cancer, smooth muscle cancer, soft tissue cancer, somatostatin-secreting tumor, spine cancer, squamous cell carcinoma, striated muscle cancer, submesothelial cancer, superficial spreading melanoma, T cell leukemia, tongue cancer, undifferentiated carcinoma, ureter cancer, urethra cancer, urinary bladder cancer, urinary system cancer, uterine cervix cancer, uterine corpus cancer, uveal melanoma, vaginal cancer, verrucous carcinoma, VIPoma, vulva cancer, well-differentiated carcinoma, or Wilms tumor.

[00147] In some other embodiments, the cancer to be treated is a non-Hodgkin's lymphoma, such as a B-cell lymphoma or a T-cell lymphoma. In certain embodiments, the non-Hodgkin's lymphoma is a B-cell lymphoma, such as a diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, follicular lymphoma, small lymphocytic lymphoma, mantle cell lymphoma, marginal zone B-cell lymphoma, extranodal marginal zone B-cell lymphoma, nodal marginal zone B-cell lymphoma, splenic marginal zone B-cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, or primary central nervous system (CNS) lymphoma. In certain other embodiments, the non-Hodgkin's lymphoma is a T-cell lymphoma, such as a precursor T-lymphoblastic lymphoma, peripheral T-cell lymphoma, cutaneous T-cell lymphoma, angioimmunoblastic T-cell lymphoma, extranodal natural killer/T-cell lymphoma, enteropathy type T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, anaplastic large cell lymphoma, or peripheral T-cell lymphoma.

[00148] In exemplary embodiments, compositions of the invention are used to treat any one of the group of an adenocarcinoma, a leukemia, a lymphoma, a melanoma, a myeloma, a sarcoma or a teratocarcinoma in subjects in need thereof. In further embodiments, compositions of the invention are used to treat subjects suffering from a cancer in one or more of adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid or uterus.

4. Administration for cancer

[00149] In some embodiments, the composition increasing expression of ChAT in T cells is delivered systemically, locally, or both. In some embodiments the composition increasing expression of ChAT in T cells is delivered alone systemically, locally, or both. In other embodiments the composition increasing expression of ChAT in T cells is delivered with one or more anti-cancer therapeutic agents, with the composition increasing expression of ChAT in T cells and the at least one of the one or more anti-cancer therapeutic agents being delivered locally. In other embodiments, the composition increasing expression of ChAT in T cells is delivered with one or more anti-cancer therapeutic agents, with the composition increasing expression of ChAT in T cells being delivered locally and the at least one of the one or more anti-cancer therapeutic agents being delivered systemically. In other embodiments, the composition increasing expression of ChAT in T cells is delivered with one or more anti-cancer therapeutic agents with the composition increasing expression of ChAT in T cells being delivered systemically and the at least one of the one or more anti-cancer therapeutic agents being delivered locally. In other embodiments, the composition increasing expression of ChAT in T cells is delivered with one or more anti-cancer therapeutic agents with the composition increasing expression of ChAT in T cells and at least one of the one or more anti-cancer therapeutic agents both being delivered systemically.

[00150] In some embodiments, the combination treatments for cancer are administered at the same time. In other embodiments, the combination treatments for cancer are administered at different times.

B. Viral infection

1. Modulating ChAT in T cells expression in T cells to treat viral infection

[00151] In some embodiments, the present invention provides methods of treating viral infection in a subject, the method including administering to the subject a composition increasing ChAT expression in T cells. In some embodiments, increasing expression of ChAT in T cells increases the ability of the body to promote viral clearance. In some embodiments, an increase in ChAT expression in T cells increases vasodilation, resulting in migration of anti-viral T cells into infected tissues, which helps

to restore anti-viral control. In some embodiments, an increase in ChAT expression in T cells sustains anti-viral responses.

[00152] In some embodiments, increasing expression of ChAT in T cells increases the ability of a therapeutic agent to reach a virally infected tissue. In other embodiments, increasing expression of ChAT in T cells increases leucocytes in a virally infected tissue. In other embodiments, increasing ChAT expression in T cells increases lymphocytes in a virally infected tissue. In yet other embodiments, increasing ChAT expression in T cells increases T cells in a virally infected tissue. In other embodiments, increasing ChAT expression in T cells increases CD4+ T cells in a virally infected tissue. In other embodiments, increasing ChAT expression in T cells increases CD4+CXCR5+PD-1+ T cells in a virally infected tissue. In other embodiments, increasing ChAT expression in T cells increases CD8+ T cells in a virally infected tissue. In some embodiments, increasing ChAT expression in T cells increases B cells in a virally infected tissue. In other embodiments, increasing ChAT expression in T cells increases CD11b+ cells in a virally infected tissue. In other embodiments, increasing ChAT expression in T cells increases dendritic cells in a virally infected tissue. In other embodiments, increasing ChAT expression in T cells increases neutrophil cells in a virally infected tissue. In other embodiments, increasing expression of ChAT in T cells increases the number of virally infected tissue infiltrating lymphocyte cells present in a virally infected tissue.

2. Modulating ChAT expression in T cells in combination with other therapeutic agents for viral infection

[00153] In some embodiments, methods or compositions that modulate ChAT expression in T cells can be combined with other therapeutic agents for the treatment of viral infection.

[00154] In some embodiments, the composition for treating viral infection may be combined with one or more anti-viral therapeutic agents to enhance an anti-viral response. Examples of anti-viral treatments include interferon compounds, acyclovir, adefovir, abacavir, amprenavir, amantadine, ampligen, arbidol, atazanavir, atipla, balavir, combivir, cidofovir, dolutegravir, darunavir, delavirdine, docosanol, didanosine, edoxudine, efavirenz, emtricitabine, enfuvirtide, entecavir, ecoliever, fomivirsen sodium, fosamprenavir, fosfonet, ibacitabine, imunovir, idoxuridine, imiquimod, indinavir,

inosine, integrase inhibitor, interferon, lopinavir, loviride, maraviroc, moroxydine, methisazone, nevirapine, nexavir, nitazoxanide, nucleoside analogs, norvir, oseltamivir, peginterferon alfa-2a, penciclovir, peramivir, pleconaril, podophyllotoxin, protease inhibitors, raltegravir, reverse transcriptase inhibitors, ribavirin, rimantadine, ritonavir, pyramide, saquinavir, sofosbuvir, stavudine, telaprevir, tenofovir, tipranavir, trifluridine, trizivir, tromantadine, truvada, valganciclovir, vicriviroc, zanamivir, dipivoxil, adenine, arabinoside, famciclovir, ganciclovir, lopinavir, ritonavir, lamivudine, nelfinavir mesylate, amivudine, lobucavir, zidovudine, indinavir, nevirapine, delavirdine, saquinavir, efavirenz, foscarnet, n-docosanol, oseltamivir, valacyclovir, palivizumab, doxuridine, miquimod, vidarabine, trifuridine, ritonavir, neuraminidase inhibitor, disoproxil fumarate and zalcitabine.

[00155] In some embodiments, the composition modulating ChAT expression in T cells is conjugated to another anti-cancer therapeutic agent. Conjugation can occur through any method known to a person skilled in the art. In some embodiments, conjugation of the composition modulating ChAT expression in T cells may be to an antibody. In some embodiments, the composition modulating ChAT expression in T cells may be conjugated to a small molecule. In some embodiments, the composition modulating ChAT expression in T cells may be a component of an antibody drug conjugate. In other embodiments the composition modulating ChAT expression in T cells may be conjugated to a peptide. In some embodiments, the composition modulating ChAT expression in T cells may be a component of a peptide drug conjugate. In some embodiments, the composition modulating ChAT expression in T cells may be conjugated to an oligonucleotide.

3. Viral infections to be treated

[00156] The present disclosure provides methods of treating viral infection. In some embodiments, the viral infection to be treated is responsive to existing anti-viral treatments. In some embodiments, the viral infection to be treated is non-responsive to existing anti-viral treatments.

[00157] In some embodiments, the viral infection to be treated includes, but is not limited to a group consisting of DNA viruses, RNA viruses, hepadnaviruses, paramyxoviruses, orthomyxoviruses, rabies virus, influenza virus, rhino virus, adenovirus, west nile virus, dengue virus, vesicular stomatitis virus, venezuelan equine

encephalitis virus, pichinde virus, coxsackie virus, polio virus, vaccinia virus, HIV-1, HIV-2, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, herpes simplex virus type 1, herpes simplex virus type 2, varicella-zoster virus, cytomegalovirus, Epstein Barr virus, human herpes virus type 6, human herpes virus type 7 and human herpes virus type 8, parainfluenza viruses, rift valley virus, lassa fever virus, ebola virus, yellow fever, papilloma viruses, pox viruses, smallpox virus, lymphocytic choriomeningitis virus, HTLV I, HTLV II, and respiratory syncytial viruses.

[00158] In further embodiments, compositions of the invention are used to treat subjects suffering from a viral infection in one or more of adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid or uterus.

4. Administration for viral infection

[00159] In some embodiments, the composition increasing expression of ChAT in T cells is delivered systemically, locally, or both. In some embodiments the composition increasing expression of ChAT in T cells is delivered alone systemically, locally, or both. In other embodiments the composition increasing expression of ChAT in T cells is delivered with one or more anti-viral therapeutic agents, with the composition increasing expression of ChAT in T cells and the at least one of the one or more anti-viral therapeutic agents being delivered locally. In other embodiments, the composition increasing expression of ChAT in T cells is delivered with one or more anti-viral therapeutic agents, with the composition increasing expression of ChAT in T cells being delivered locally and the at least one of the one or more anti-viral therapeutic agents being delivered systemically. In other embodiments, the composition increasing expression of ChAT in T cells is delivered with one or more anti-viral therapeutic agents with the composition increasing expression of ChAT in T cells being delivered systemically and the at least one of the one or more anti-viral therapeutic agents being delivered locally. In other embodiments, the composition increasing expression of ChAT in T cells is delivered with one or more anti-viral therapeutic agents with the

composition increasing expression of ChAT in T cells and at least one of the one or more anti-viral therapeutic agents both being delivered systemically.

[00160] In some embodiments, the combination treatments for viral infections are administered at the same time. In other embodiments, the combination treatments for viral infections are administered at different times.

IV. Treating cancer/viral infection by determining ChAT expression levels and applying a vasodilator

A. Obtaining the sample from a subject

[00161] In some embodiments, the sample taken from a subject is peripheral blood. In some embodiments, the sample taken from a subject is isolated from peripheral blood. Some examples of sample types that can be isolated from peripheral blood include plasma, serum, cell pellet, isolated peripheral blood mononuclear cells, any specific cell types, proteins, DNA, and RNA. In other embodiments, the sample taken from a subject is a biopsy from tissue. Biopsies can be taken using any method known to a person skilled in the art, including but not limited to core needle biopsies, fine needle biopsies, punch biopsies, and other surgical biopsies that are incisional or excisional. Samples may be processed fresh, frozen, or preserved in any method known to a person skilled in the art.

B. Measuring the expression of ChAT present in the sample

[00162] In some embodiments, the level of expression of ChAT is measured in T cells. In other embodiments, total ChAT expression may be measured along with methods quantifying the number or percentage of cell types in a given sample. Examples of methods of quantifying the number or percentage of cell types in a given sample include flow cytometry or hemocytometers. Quantification may be relative to a normal sample, where the level of ChAT expression in T cells is known. In some embodiments, the tools used to measure the level of choline acetyltransferase in the sample can be through immunoassay methods. Methods to measure ChAT include any methods used or described by those skilled in the art. In some embodiments, methods to measure the level of ChAT in a sample include but are not limited to flow cytometry, mass spectrometry, mass cytometry, enzyme-linked immunosorbent assays, enzyme-

linked immunospot assays, immunofluorescence assays, immunohistochemistry, and radioimmunoassays. ChAT can be measured in specific cell types including but not limited to leucocytes, lymphocytes, and T cells. In some embodiments, the tools used to measure the level of ChAT in the sample include polymerase chain reaction (PCR) methods. Examples of PCR methods include real-time PCR, quantitative PCR, and digital droplet PCR. In some embodiments, the tools used to measure the level of ChAT in the sample include microarray methods.

[00163] In other embodiments, products upstream or downstream of ChAT expression in T cells may be used as surrogate markers for ChAT expression in T cells when they are appropriately correlated. Examples of potential surrogate markers include but are not limited to acetyl-CoA, choline, ACh, IL-21, and pSTAT3. The ratio of acetyl-CoA to ACh or choline to ACh or both may be used instead of measuring ChAT due to ChAT's role as the lone catalyst for the production of ACh. IL-21 may be used as a surrogate marker as lack of IL-21 signaling reduces ChAT expression in CD4⁺ and CD8⁺ T cells. pSTAT3 is a known downstream activation marker of IL-21 function for T cells involved in the activation of ChAT in T cells, so pSTAT3 expression could also be used as a surrogate marker for ChAT expression.

C. Assessing/diagnosing whether a patient (also referred to herein as "subject") has low ChAT expression in T cells

[00164] In some embodiments, a patient is identified as having low ChAT expression in T cells if the patient's expression levels are 99% of normal ChAT expression in T cells. In some embodiments a patient with low ChAT expression in T cells exhibits 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, of normal expression of ChAT. In some embodiments, low ChAT expression in T cells is between 0-10%, 0-20%, 0-30%, 0-40%, 0-50%, 0-60%, 0-70%, 0-80%, 0-90% of normal ChAT expression in T cells. Normal expression of ChAT is determined by a reference sample or a control. In some embodiments, a reference sample or control is obtained from a healthy individual who is not the subject being assessed for low ChAT expression in T cells.

D. Administering a pharmaceutically effective amount of a vasodilator

1. Vasodilator alone

[00165] In some embodiments, vasodilators comprising the present invention include, but are not limited to: angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, and nitrates.

[00166] In some embodiments, angiotensin converting enzyme inhibitor refers to benazepril, captopril, enalapril, fosinopril, Lisinopril, moexipril, perindopril, quinapril, Ramipril, trandolapril.

[00167] In some embodiments, angiotensin receptor blockers refers to azilsartan, candesartan, eprosartan, irbesartan, telmisartan, valsartan, losartan, olmesartan.

[00168] In some embodiments, calcium channel blockers refers to amlodipine, clevidipine, diltazem, felodipine, isradipine, nicardipine, nimodipine, nisoldipine, verapamil.

[00169] In some embodiments, nitrites refers to nitroglycerin, isosorbide mononitrate, isosorbide dinitrate, hydralazine, fenoldopam, nitroprusside.

[00170] In further embodiments, vasodilators include minoxidil, alprostadil, riociguat, nesiritide, nitric oxide, oxygen, sildenafil, tadalafil, bosentan.

[00171] In some embodiments, vasodilators are tablets. In some embodiments, vasodilators are capsules. In some embodiments vasodilators are injections. In some embodiments vasodilators are topical gels. In some embodiments vasodilators are sprays. In some embodiments, vasodilators are patches for the skin.

[00172] In some embodiments, vasodilators include arginine, bencyclane fumarate, benzyl nicotinate, buphenine hydrochloride, ciclonicate, cyclandelate, ethyl nicotinate, hepronicate, hexyl nicotinate, hydralazine, inositol nicotinate, isoxsuprine hydrochloride, methyl nicotinate, minoxidol, naftidrofuryl oxalate, nicametate citrate, niceritrol, nicoboxil, nicofuranose, nicotiny alcohol, nicotiny alcohol tartrate, nitric oxide, nitroglycerin, nonivamide, oxpentifylline, papaverine, papaveroline, pentifylline, peroxyxynitrite, pinacidil, sodium nitroprusside, suloctidil, teasuprine, thymoxamine hydrochloride, tolazoline, vitamin E nicotinate, and xanthinol nicotinate. Centrally acting vasomodulatory agents include clonidine, guanabenz, and methyl dopa. Alpha-adrenoceptor blocking agents include indoramin, phenoxybenzamine, phentolamine, and prazosin. Adrenergic neuron blocking agents include bedmidine, debrisoquine, and guanethidine. ACE inhibitors include benazepril, captopril, cilazapril, enalapril, fosinopril, lisinopril, perindopril, quinapril, and ramipril. Ganglion-blocking agents include

pentolinium and trimetaphan. Calcium channel blockers include amlodipine, diltiazem, felodipine, isradipine, nicardipine, nifedipine, nimodipine, and verapamil. Prostaglandins including: prostacyclin, thromboxane A₂, leukotrienes, PGA, PGA₁, PGA₂, PGE₁, PGE₂, PGD, PGG, and PGH. Angiotensin II analogs include saralasin.

[00173] In further aspects, compositions of the invention may include any of the vasodilators discussed herein along with additives and pharmaceutically acceptable carriers. As used herein, "pharmaceutically acceptable carrier" includes any material, which when combined with the conjugate retains the conjugates' activity and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well-known conventional methods.

2. Vasodilator in combination with agents to treat cancer

[00174] In some embodiments, the method further comprises administering one or more anti-cancer therapeutic agents with the vasodilator. Examples of anti-cancer therapeutic agents to combine with vasodilators are provided in Section III above. In further embodiments, the method further comprises administering at least one checkpoint inhibitor. In further embodiments, the at least one checkpoint inhibitor is a member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a Tim-3 inhibitor, and a Lag-3 inhibitor. These checkpoint inhibitors serve to increase tumor cell killing and clearance. In further embodiments, the vasodilator is delivered systemically, locally, or both. In some embodiments the vasodilator is delivered alone systemically, locally, or both. In other embodiments the vasodilator is delivered with one or more anti-cancer therapeutic agents, with the vasodilator and the at least one of the one or more anti-cancer therapeutic agents being delivered locally. In other embodiments, the vasodilator is delivered with one or more anti-cancer therapeutic agents, with the vasodilator being delivered locally and the at least one of the one or more anti-cancer therapeutic agents being delivered

systemically. In other embodiments, the vasodilator is delivered with one or more anti-cancer therapeutic agents with the vasodilator being delivered systemically and the at least one of the one or more anti-cancer therapeutic agents being delivered locally. In other embodiments, the vasodilator is delivered with one or more anti-cancer therapeutic agents with the vasodilator and at least one of the one or more anti-cancer therapeutic agents both being delivered systemically.

[00175] In some embodiments, the vasodilator is conjugated to another anti-cancer therapeutic agent. Conjugation can occur through any method known to a person skilled in the art. In some embodiments, conjugation of a vasodilator may be to an antibody. In some embodiments, the vasodilator may be conjugated to a small molecule. In some embodiments, the vasodilator may be a component of an antibody drug conjugate. In other embodiments the vasodilator may be conjugated to a peptide. In some embodiments, the vasodilator may be a component of a peptide drug conjugate. In some embodiments, the vasodilator may be conjugated to an oligonucleotide.

[00176] In some embodiments, the vasodilator is coated on T cells *ex-vivo* and the T cells coated by the vasodilator are administered to the subject. T cells can be taken from the subject to be treated or from a donor subject. In some embodiments, the T cells are autologous. In other embodiments, the T cells are non-autologous. In some embodiments, the T cells are allogenic. In other embodiments, the T cells are antigen-specific. In other embodiments, the T cells are tumor infiltrating lymphocytes. T cells can be engineered to modulate other targets along with being coated with a vasodilator. Vasodilators can be engineered to bind to the T cells *in-vitro*. Vasodilators can be pre-incubated *in-vitro* for any amount of time before being transferred into a patient to allow coating of T cells. Vasodilators may be pre-incubated with any other number of factors which allows the T cells to preferentially kill cancer cells. In some embodiments, T cells may be activated before being coated with the vasodilator. In some embodiments, T cells may be coated with the vasodilator while being activated. In some embodiments, the T cells may be coated with the vasodilator and then be activated. In some embodiments, the T cells may not be activated but coated with a vasodilator. In some embodiments, T cells may be activated by the vasodilator conjugated to an anti-cancer therapeutic agent. In some embodiments, vasodilator coating allows for increased delivery of T cells to a tumor. In some embodiments, the vasodilator increases tumor infiltrating lymphocytes in a tumor.

3. Vasodilator in combination with agents to treat viral infection

[00177] In some embodiments, the method further comprises administering one or more anti-viral therapeutic agents with the vasodilator. Examples of anti-viral therapeutic agents to combine with vasodilators are provided in Section III above.

[00178] In further embodiments, the vasodilator is delivered systemically, locally, or both. In some embodiments the vasodilator is delivered by itself systemically, locally, or both. In other embodiments the vasodilator is delivered with one or more anti-viral therapeutic agents, with the vasodilator and the at least one of the one or more anti-viral therapeutic agents being delivered locally. In other embodiments, the vasodilator is delivered with one or more anti-viral therapeutic agents, with the vasodilator being delivered locally and the at least one of the one or more anti-viral therapeutic agents being delivered systemically. In other embodiments, the vasodilator is delivered with one or more anti-viral therapeutic agents with the vasodilator being delivered systemically and the at least one of the one or more anti-viral therapeutic agents being delivered locally. In other embodiments, the vasodilator is delivered with one or more anti-viral therapeutic agents with the vasodilator and at least one of the one or more anti-viral therapeutic agents both being delivered systemically.

[00179] In some embodiments, the vasodilator is conjugated to another anti-viral therapeutic agent. Conjugation can occur through any method known to a person skilled in the art. In some embodiments, conjugation of a vasodilator may be to an antibody. In some embodiments, the vasodilator may be conjugated to a small molecule. In some embodiments, the vasodilator may be a component of an antibody drug conjugate. In other embodiments the vasodilator may be conjugated to a peptide. In some embodiments, the vasodilator may be a component of a peptide drug conjugate. In some embodiments, the vasodilator may be conjugated to an oligonucleotide.

[00180] In some embodiments, the vasodilator is coated on T cells *ex-vivo* and the T cells coated by the vasodilator are administered to the subject. T cells can be taken from the subject to be treated or from a donor subject. In some embodiments, the T cells are autologous. In other embodiments, the T cells are non-autologous. In some embodiments, the T cells are allogenic. In other embodiments, the T cells are antigen-specific. In other embodiments, the T cells are tumor infiltrating lymphocytes. T cells

can be engineered to modulate other targets along with being coated with a vasodilator. Vasodilators can be engineered to bind to the T cells in-vitro. Vasodilators can be pre-incubated in-vitro for any amount of time before being transferred into a patient to allow coating of T cells. Vasodilators may be pre-incubated with any other number of factors which allows the T cells to preferentially kill cancer cells. In some embodiments, T cells may be activated before being coated with the vasodilator. In some embodiments, T cells may be coated with the vasodilator while being activated. In some embodiments, the T cells may be coated with the vasodilator and then be activated. In some embodiments, the T cells may not be activated but coated with a vasodilator. In some embodiments, T cells may be activated by the vasodilator conjugated to an anti-viral therapeutic agent. In some embodiments, vasodilator coating allows for increased delivery of T cells to a virally infected tissue. In some embodiments, the vasodilator increases virus-specific T cells in a virally infected tissue.

V. Treating cancer/viral infection by determining ChAT activity levels and applying a vasodilator

A. Obtaining the sample from a subject

[00181] In some embodiments, the sample taken from a subject is peripheral blood. In some embodiments, the sample taken from a subject is isolated from peripheral blood. Some examples of sample types that can be isolated from peripheral blood include plasma, serum, cell pellet, isolated peripheral blood mononuclear cells, any specific cell types, proteins, DNA, and RNA. In other embodiments, the sample taken from a subject is a biopsy from tissue. Biopsies can be taken using any method known to a person skilled in the art, including but not limited to core needle biopsies, fine needle biopsies, punch biopsies, and other surgical biopsies that are incisional or excisional. Samples may be processed fresh, frozen, or preserved in any method known to a person skilled in the art.

B. Measuring the activity of ChAT present in the sample

[00182] Previous studies have identified mutations in ChAT that lower ChAT activity in humans (Ohno K et al., "Choline acetyltransferase mutations cause myasthenic syndrome associated with episodic apnea in humans". *Proceedings of the National*

Academy of Sciences. (2001) 98 (4): 2017–2022. doi:10.1073/pnas.98.4.2017.) By measuring ChAT activity, additional subjects that may have normal expression of ChAT but lower functionality, may be identified for treatment with a vasodilator. In some embodiments, the level of activity of ChAT is measured in T cells. In other embodiments, due to the difficulty and cost associated with determining ChAT activity in T cells, total ChAT activity may be measured. In some embodiments, the tools used to measure the level of choline acetyltransferase in the sample can be through immunoassay methods. Methods to measure ChAT include any methods used or described by those skilled in the art. In some embodiments, methods to measure the level of ChAT in a sample include but are not limited to flow cytometry, mass spectrometry, mass cytometry, enzyme-linked immunosorbent assays, enzyme-linked immunospot assays, immunofluorescence assays, immunohistochemistry, and radioimmunoassays. ChAT can be measured in specific cell types including but not limited to leucocytes, lymphocytes, and T cells.

[00183] In some embodiments, the tools used to measure the level of ChAT in the sample include polymerase chain reaction (PCR) methods.

[00184] In some embodiments, the tools used to measure the level of ChAT in the sample include microarray methods.

[00185] In other embodiments, products upstream or downstream of ChAT activity in T cells may be used as surrogate markers for ChAT activity in T cells when they are appropriately correlated. Examples of potential surrogate markers include but are not limited acetyl-CoA, choline, ACh, IL-21, and pSTAT3. The ratio of acetyl-CoA to ACh or choline to ACh or both may be used instead of measuring ChAT due to ChAT's role as the lone catalyst for the production of ACh. IL-21 may be used as a surrogate marker as lack of IL-21 signaling reduces ChAT expression in CD4+ and CD8+ T cells. pSTAT3 is a known downstream activation marker of IL-21 function for T cells involved in the activation of ChAT in T cells, so pSTAT3 expression could also be used as a surrogate marker for ChAT activity.

C. Assessing whether a patient has low ChAT activity in T cells

[00186] In some embodiments, a patient is deemed to have low ChAT activity in T cells if that activity is 95% or less than normal ChAT activity. In some embodiments such low activity is 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%,

84%, 83%, 82%, 81%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, or 5% of normal activity of ChAT. In some embodiments, low ChAT activity in T cells is between 0-10%, 0-20%, 0-30%, 0-40%, 0-50%, 0-60%, 0-70%, 0-80%, 0-90% of normal ChAT activity in T cells. Normal activity of ChAT can be determined by a reference sample or a control. In some embodiments, a reference sample or control is obtained from a healthy individual who is not the subject being assessed for low ChAT activity.

D. Administering a pharmaceutically effective amount of a vasodilator

1. Vasodilator alone

[00187] In some embodiments, vasodilators of the present invention include, but are not limited to: angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, and nitrates.

[00188] In some embodiments, angiotensin converting enzyme inhibitor refers to benazepril, captopril, enalapril, fosinopril, Lisinopril, moexipril, perindopril, quinapril, Ramipril, trandolapril.

[00189] In some embodiments, angiotensin receptor blockers refers to azilsartan, candesartan, eprosartan, irbesartan, telmisartan, valsartan, losartan, olmesartan.

[00190] In some embodiments, calcium channel blockers refers to amlodipine, clevidipine, diltazem, felodipine, isradipine, nicardipine, nimodipine, nisoldipine, verapamil.

[00191] In some embodiments, nitrites refers to nitroglycerin, isosorbide mononitrate, isosorbide dinitrate, hydralazine, fenoldopam, nitroprusside.

[00192] In further embodiments, vasodilators include minoxidil, alprostadil, riociguat, nesiritide, nitric oxide, oxygen, sildenafil, tadalafil, bosentan.

[00193] In some embodiments, vasodilators are tablets. In some embodiments, vasodilators are capsules. In some embodiments vasodilators are injections. In some embodiments vasodilators are topical gels. In some embodiments vasodilators are sprays. In some embodiments, vasodilators are patches for the skin.

[00194] In some embodiments, vasodilators include arginine, bencyclane fumarate, benzyl nicotinate, buphenine hydrochloride, ciclonicate, cyclandelate, ethyl nicotinate, hepronicate, hexyl nicotinate, hydralazine, inositol nicotinate, isoxsuprine hydrochloride, methyl nicotinate, minoxidol, naftidrofuryl oxalate, nicametate citrate, niceritrol,

nicoboxil, nicofuranose, nicotiny alcohol, nicotiny alcohol tartrate, nitric oxide, nitroglycerin, nonivamide, oxpentifylline, papaverine, papaveroline, pentifylline, peroxyxynitrite, pinacidil, sodium nitroprusside, suloctidil, teasuprine, thymoxamine hydrochloride, tolazoline, vitamin E nicotinate, and xanthinol nicotinate. Centrally acting vasomodulatory agents include clonidine, guanabenz, and methyl dopa. Alpha-adrenoceptor blocking agents include indoramin, phenoxybenzamine, phentolamine, and prazosin. Adrenergic neuron blocking agents include bedmidine, debrisoquine, and guanethidine. ACE inhibitors include benazepril, captopril, cilazapril, enalapril, fosinopril, lisinopril, perindopril, quinapril, and ramipril. Ganglion-blocking agents include pentolinium and trimetaphan. Calcium channel blockers include amlodipine, diltiazem, felodipine, isradipine, nicardipine, nifedipine, nimodipine, and verapamil. Prostaglandins including: prostacyclin, thromboxane A₂, leukotrienes, PGA, PGA₁, PGA₂, PGE₁, PGE₂, PGD, PGG, and PGH. Angiotensin II analogs include saralasin.

[00195] In further aspects, compositions of the invention may include any of the vasodilators discussed herein along with additives and pharmaceutically acceptable carriers. As used herein, "pharmaceutically acceptable carrier" includes any material, which when combined with the conjugate retains the conjugates' activity and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well-known conventional methods.

[00196]

2. Vasodilator in combination with agents to treat cancer

[00197] In some embodiments, the method further comprises administering one or more anti-cancer therapeutic agents with the vasodilator. Examples of anti-cancer therapeutic agents to combine with vasodilators are provided in Section III above. In further embodiments, the method further comprises administering at least one checkpoint inhibitor. In further embodiments, the at least one checkpoint inhibitor is a

member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a Tim-3 inhibitor, and a Lag-3 inhibitor. These checkpoint inhibitors serve to increase tumor cell killing and clearance. In further embodiments, the vasodilator is delivered systemically, locally, or both. In some embodiments the vasodilator is delivered alone systemically, locally, or both. In other embodiments the vasodilator is delivered with one or more anti-cancer therapeutic agents, with the vasodilator and the at least one of the one or more anti-cancer therapeutic agents being delivered locally. In other embodiments, the vasodilator is delivered with one or more anti-cancer therapeutic agents, with the vasodilator being delivered locally and the at least one of the one or more anti-cancer therapeutic agents being delivered systemically. In other embodiments, the vasodilator is delivered with one or more anti-cancer therapeutic agents with the vasodilator being delivered systemically and the at least one of the one or more anti-cancer therapeutic agents being delivered locally. In other embodiments, the vasodilator is delivered with one or more anti-cancer therapeutic agents with the vasodilator and at least one of the one or more anti-cancer therapeutic agents both being delivered systemically.

[00198] In some embodiments, the vasodilator is conjugated to another anti-cancer therapeutic agent. Conjugation can occur through any method known to a person skilled in the art. In some embodiments, conjugation of a vasodilator may be to an antibody. In some embodiments, the vasodilator may be conjugated to a small molecule. In some embodiments, the vasodilator may be a component of an antibody drug conjugate. In other embodiments the vasodilator may be conjugated to a peptide. In some embodiments, the vasodilator may be a component of a peptide drug conjugate. In some embodiments, the vasodilator may be conjugated to an oligonucleotide.

[00199] In some embodiments, the vasodilator is coated on T cells *ex-vivo* and the T cells coated by the vasodilator are administered to the subject. T cells can be taken from the subject to be treated or from a donor subject. In some embodiments, the T cells are autologous. In other embodiments, the T cells are non-autologous. In some embodiments, the T cells are allogenic. In other embodiments, the T cells are antigen-specific. In other embodiments, the T cells are tumor infiltrating lymphocytes. T cells can be engineered to modulate other targets along with being coated with a vasodilator. Vasodilators can be engineered to bind to the T cells *in-vitro*. Vasodilators can be pre-incubated *in-vitro* for any amount of time before being transferred into a patient to allow

coating of T cells. Vasodilators may be pre-incubated with any other number of factors which allows the T cells to preferentially kill cancer cells. In some embodiments, T cells may be activated before being coated with the vasodilator. In some embodiments, T cells may be coated with the vasodilator while being activated. In some embodiments, the T cells may be coated with the vasodilator and then be activated. In some embodiments, the T cells may not be activated but coated with a vasodilator. In some embodiments, T cells may be activated by the vasodilator conjugated to an anti-cancer therapeutic agent. In some embodiments, vasodilator coating allows for increased delivery of T cells to a tumor. In some embodiments, the vasodilator increases tumor infiltrating lymphocytes in a tumor.

3. Vasodilator in combination with agents to treat viral infection

[00200] In some embodiments, the method further comprises administering one or more anti-viral therapeutic agents with the vasodilator. Examples of anti-viral therapeutic agents to combine with vasodilators are provided in Section III above.

[00201] In further embodiments, the vasodilator is delivered systemically, locally, or both. In some embodiments the vasodilator is delivered by itself systemically, locally, or both. In other embodiments the vasodilator is delivered with one or more anti-viral therapeutic agents, with the vasodilator and the at least one of the one or more anti-viral therapeutic agents being delivered locally. In other embodiments, the vasodilator is delivered with one or more anti-viral therapeutic agents, with the vasodilator being delivered locally and the at least one of the one or more anti-viral therapeutic agents being delivered systemically. In other embodiments, the vasodilator is delivered with one or more anti-viral therapeutic agents with the vasodilator being delivered systemically and the at least one of the one or more anti-viral therapeutic agents being delivered locally. In other embodiments, the vasodilator is delivered with one or more anti-viral therapeutic agents with the vasodilator and at least one of the one or more anti-viral therapeutic agents both being delivered systemically.

[00202] In some embodiments, the vasodilator is conjugated to another anti-viral therapeutic agent. Conjugation can occur through any method known to a person skilled in the art. In some embodiments, conjugation of a vasodilator may be to an antibody. In some embodiments, the vasodilator may be conjugated to a small molecule. In some embodiments, the vasodilator may be a component of an antibody

drug conjugate. In other embodiments the vasodilator may be conjugated to a peptide. In some embodiments, the vasodilator may be a component of a peptide drug conjugate. In some embodiments, the vasodilator may be conjugated to an oligonucleotide.

[00203] In some embodiments, the vasodilator is coated on T cells *ex-vivo* and the T cells coated by the vasodilator are administered to the subject. T cells can be taken from the subject to be treated or from a donor subject. In some embodiments, the T cells are autologous. In other embodiments, the T cells are non-autologous. In some embodiments, the T cells are allogenic. In other embodiments, the T cells are antigen-specific. In other embodiments, the T cells are tumor infiltrating lymphocytes. T cells can be engineered to modulate other targets along with being coated with a vasodilator. Vasodilators can be engineered to bind to the T cells *in-vitro*. Vasodilators can be pre-incubated *in-vitro* for any amount of time before being transferred into a patient to allow coating of T cells. Vasodilators may be pre-incubated with any other number of factors which allows the T cells to preferentially kill cancer cells. In some embodiments, T cells may be activated before being coated with the vasodilator. In some embodiments, T cells may be coated with the vasodilator while being activated. In some embodiments, the T cells may be coated with the vasodilator and then be activated. In some embodiments, the T cells may not be activated but coated with a vasodilator. In some embodiments, T cells may be activated by the vasodilator conjugated to an anti-viral therapeutic agent. In some embodiments, vasodilator coating allows for increased delivery of T cells to a virally infected tissue. In some embodiments, the vasodilator increases virus-specific T cells in a virally infected tissue.

Examples

Example 1: ChAT expression is robustly induced in adaptive immune cells by viral infection and sustained during chronic infection.

[0101] ChAT–GFP reporter mice (Jackson Labs) were infected with the rapidly-cleared Armstrong strain of lymphocytic choriomeningitis virus (LCMV-Arm, propagated in house). Using flow cytometry, spleens of mice at 8 days post-infection showed a massive increase in ChAT-GFP expression in CD4⁺ T cells (Fig. 1A; anti-CD4-PECy7, Biolegend Cat. #100548, clone RM4-5) and in CD8⁺ T cells (Fig. 1B; anti-CD8-eFlour450, Ebioscience Cat. #48-0081-82). ChAT-GFP expression in splenic virus-specific T cells then rapidly declined as LCMV-Arm was cleared (Fig 1C-H). In contrast,

ChAT expression was retained by virus-specific CD4⁺ and CD8⁺ T cells in mice infected with the persistent clone-13 strain of LCMV (LCMV-C113, propagated in house) (Figure 1C-H).

[0102] ChAT was also induced in responding splenic B220⁺ PD-1⁺ GL-7⁺ germinal center (GC) B cells of mice infected with either LCMV strain, although this induction was lower in LCMV-C113-infected animals and ChAT was not retained in this population during persistent infection (Fig. 1L, 1J). After evaluating the spatial location of ChAT-GFP⁺ cells in the spleen using IHC, it was discovered that the ChAT-GFP⁺ cells were distributed throughout the spleen of both LCMV-Arm and LCMV-C1-13 infected mice, with dense concentrations in the white pulp (Fig. 1K).

[0103] Pooled splenocytes from n=5 ChAT-GFP mice infected 8 days previously with LCMV-C1-13 were sorted to obtain CD4⁺ GFP⁺, CD4⁺ GFP⁻, CD8⁺ GFP⁺, and CD8⁺ GFP⁻ populations. RNA was isolated from the cells, and expression of ChAT and RSP9 was evaluated by RT-PCR. ChAT expression in CD4⁺ and CD8⁺ populations was normalized to the expression in the relevant sorted GFP⁻ population (FIG. 1L). The data are a composite of 2 experimental cohorts.

Example 2: ChAT expression is associated with a Tfh phenotype in virus specific CD4⁺ T cells and with PD-1 expression in virus-specific CD8⁺ T cells.

[0104] This example provides data generated through tetramer staining (NIH Tetramer Core) and flow cytometry which characterizes the phenotype of ChAT⁺ CD4⁺ and CD8⁺ T cells in LCMV-infected ChAT-GFP reporter mice. Within the virus-specific CD4⁺ compartment, ChAT expression was strongest in CXCR5⁺ PD-1^{hi} T follicular helper cells (Tfh), although robust expression was still detected in PD-1^{lo} populations (Fig. 2A). During persistent LCMV infection, CD4⁺ T cells preferentially acquire a “Tfh-like” phenotype (Fahey et al., Viral persistence redirects CD4 T cell differentiation toward T follicular helper cells, J. Exp. Med. 2011. 208; 987-999), which may account for the enhanced retention of ChAT observed in CD4⁺ T cells during LCMV-C113 infection (Fig. 2C, D). At later time points post-infection, ChAT expression was preferentially retained by these virus-specific Tfh-like cells, regardless of viral persistence (Fig. 2B, D).

[0105] During acute infection, ChAT expression in CD8⁺ T cells did not correlate with expression of cell fate markers such as CD127 or KLRG-1 (Kaeck et al., Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to

long-lived memory cells. *Nature Immunology*, 2003. 4; 1191-1198)(Fig. 2E). During persistent LCMV-Cl13 infection, however, ChAT⁺ virus-specific CD8⁺ T cells tended to express higher levels of the inhibitory receptors PD-1, TIM-3, and LAG-3 than their ChAT-negative counterparts specific for the same LCMV epitope (Figure 2F-H; anti-PD-1-PE-Cy7, Biolegend Cat. #109110, clone RMP1-30; anti-Tim3-PE, Biolegend Cat. #119704, clone RMT3-23; anti-LAG-3-PerCPCy5.5, Biolegend Cat. # 125212, clone C9B7W), suggesting increased exhaustion. Alternatively, because expression of these inhibitory receptors is driven by T cell activation and antigen engagement (Blattman et al., Impact of epitope escape on PD-1 expression and CD8 T-cell exhaustion during chronic infection. *Journal of Virology*, 2009. 83; 4386-4394), ChAT expression during persistent infection may identify T cells that have been strongly activated.

Example 3: ChAT expression is driven by IL-21/Stat3 signaling in CD8⁺ T cells.

[0106] To test if specific cytokines could induce ChAT in T cells, ChAT-GFP⁺ P14 TCR transgenic T cells (P14 mice provided by P. Ohashi) were activated *in vitro* with the cognate GP33 peptide (1µg/mL) in the presence/absence of recombinant IFNβ (2500U/mL), high dose IL-2 (100U/mL), low dose IL-2 (10U/mL), IL-15 (50ng/mL), IL-7 (50ng/mL), IL-6 (30ng/mL and 10ng/mL), IL-10 (50ng/mL and 20 ng/mL), or IL-21 for 5 days, with cytokine replenishment at day 2, and measured at day 5 by flow cytometry. Only GP33 peptide plus IL-21 induced ChAT expression in P14 cells *in vitro* (FIG. 3A, FIG. 8).

[0107] Then, the contribution of IL-21 signaling to ChAT induction was evaluated by crossing ChAT-GFP reporter animals to either IL-21 receptor-deficient (IL-21R^{-/-}) mice (Jackson Labs) (Frolich et al., IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. *Science*, 2007. 324; 1576-1580), or Stat3^{flox} (Moh et al., Role of STAT3 in liver regeneration: survival, DNA synthesis, inflammatory reaction and liver mass recovery. *Laboratory investigation; a journal of technical methods and pathology*, 2007. 87, 1018-1028) (Jackson Labs) CD4-cre recombinase mice (Lee et al., A critical role for Dnmt1 and DNA methylation in T cell development function and survival. *Immunity*, 2001. 1; 763-774)(Jackson Labs), to generate ChAT-GFP IL-21R^{-/-} and ChAT-GFP Stat3^{flox/flox} Cd4-cre animals, respectively. Expression of CD4-cre occurs at the double positive stage of thymic development, deleting the floxed alleles in both CD4⁺ and CD8⁺ T cells (Lee et al., 2001).

[0108] Using flow cytometry analysis, at 8 days post-LCMV-Cl13 infection, there was a decrease in ChAT-GFP⁺ CD4⁺ and CD8⁺ T cells when IL-21R was deleted (Fig. 3B). ChAT-GFP⁺ IL-21^{+/-} mice appeared to have a mixed phenotype, consistent with the partial viral control exhibited by IL-21R^{+/-} animals (Yi et al., A vital role for interleukin-21 in the control of a chronic viral infection. *Science*, 2009. 324; 1572-1576). ChAT-GFP induction in GC B cells was not significantly decreased in IL-21R^{-/-} animals (Fig. 3B), indicating that T cells, but not responding B cells, depend on IL-21 for ChAT expression. In non-GC B cells, however, ChAT was reduced in the absence of IL-21R (Fig. 3B).

[0109] In addition to the reduced fraction of ChAT-GFP⁺ cells in IL-21 R^{-/-} mice, the cells that did express ChAT-GFP demonstrated a lower MFI for the reporter molecule, suggesting lower expression in these cells (Fig. 3C, D). Loss of Stat3 in T cells also dramatically reduced ChAT-GFP in both CD4⁺ and CD8⁺ T cells (Fig. 3E, F).

Example 4: ChAT expression in T cells sustains anti-viral responses, inhibits CD8⁺ T cell exhaustion, and promotes viral clearance.

[0110] Due to the fact that IL-21 drives ChAT expression in T cells, and ChAT is sustained during persistent immune responses, an investigation was done to see whether loss of ChAT specifically in T cells would affect antiviral responses and viral control during LCMV infection. ChAT^{flox} mice (Misgeld et al., Roles of neurotransmitter in synapse formation: development of neuromuscular junctions lacking choline acetyltransferase. *Neuron*, 2002. 36; 635-648.) (Jackson Labs) were crossed to CD4-cre mice (Lee et al., 2001) to generate ChAT^{flox/flox} cre⁻ (ChAT^{WT}) and ChAT^{flox/flox} CD4 cre⁺ (T-ChAT^{KO}) animals. These animals were then infected with LCMV-Cl13 and CD8⁺ splenocytes were determined at the indicated time points by tetramer staining and flow cytometry. While no difference in the response to acute LCMV-Arm infection was observed (Fig. 9A, B), T cell-specific loss of ChAT eroded the virus-specific CD8⁺ T cell response during persistent LCMV-Cl13 infection (Figure 4A, B). No difference was detected in the expression of the cell cycle protein Ki67 in virus-specific CD8⁺ T cells at 8 and 30 days post-infection, and in fact, higher levels were detected on days 60 and 120 post-infection in CD8⁺ T-ChAT^{KO} T cells (Fig. 9C, D).

[0111] Virus-specific cells in LCMV-Cl13-infected T-ChAT^{KO} mice showed higher PD-1, Tim-3, and LAG-3 expression than did ChAT^{WT} control cells (Fig. 9E-H), as well as a greater propensity to co-express these receptors (Fig. 4C-E). These features are

consistent with increased T cell exhaustion and dysfunction. Further evaluation of the D^b(GP276) response in T-ChAT^{KO} mice confirmed that their virus-specific CD8⁺ T cells showed impaired cytokine production when stimulated with peptide *in vitro* (Fig. 4F). Moreover, of the T-ChAT^{KO} cells that could produce IFN γ *in vitro*, a much smaller proportion could also synthesize TNF α and IL-2, measured by flow cytometry (Fig. 4F; anti-IFN γ -PE, Biolegend Cat. # 505808, clone MP6-XT22; anti-TNF α -FITC, Biolegend Cat. #506304, clone MP6-XT22; anti-IL-2, Biolegend Cat. #503810, clone JES6-5H4).

[0112] The transcription factor T-bet is important for the differentiation and effector functions of CD8⁺ T cells, and high T-bet levels repress PD-1 and LAG-3 expression to facilitate viral control (Kao et al., Transcription factor T-bet represses expression of the inhibitory receptor PD-1 and sustains virus-specific CD8⁺ T cell responses during chronic infection *Nature Immunology*, 2011. 12; 663-671). Virus specific CD8⁺ T cells from T-ChAT^{KO} mice expressed less T-bet than ChAT^{WT} virus-specific cells at 30 and 60 days post-infection (Fig. 4G, H). The transcription factor interferon regulatory factor 4 (IRF4) is likewise influenced by antigenic signaling in T cells following infection (Cretney et al., The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory cells. *Nature Immunology*, 2011. 12; 304-311) and maintains anti-viral responses during persistent LCMV infection (Grusdat et al., T cell function following infection with LCMV. *Cell death and differentiation*, 2014. 21; 1050-1060). Although a diminution in the virus-specific response in T-ChAT^{KO} mice was observed, IRF4 was elevated in their antiviral CD8⁺ T cells at 60 and 120 days post-infection (Fig. 9I, 9J; anti-IRF4-PE, Biolegend Cat. #646404, clone IRF4.3E4).

[0113] Viral titers were evaluated in the serum and organs of LCMV-CI13-infected ChAT^{WT} and T-ChAT^{KO} mice and an increase was observed in the mutants by day 30 post-infection (Fig. 4I, FIG. 9M). All ChAT^{WT} animals eventually controlled the viral titer in their serum and in all organs except the kidneys (Fig. 4I, Fig. 9M), which are known to maintain measureable viral titers following LCMV-CI13 infection (Zajac et. al., 1998). In contrast, a substantial fraction of T-ChAT^{KO} littermates could not control the virus in either their serum (Fig. 4I) or organs (Fig. 9M). Despite robust expression of ChAT in CD4 T cells (Fig. 1), no defects were observed in either anti-viral CD4⁺ T cell numbers or cytokine production (Fig. 10A, 10B).

[0114] Tfh cells are important for the development of long-lived humoral responses (Crotty, T follicular helper cell differentiation, function, and roles in disease. *Immunity*, 2014. 41; 529-542), and these cells express and retain high levels of ChAT during

infection (Fig. 2; anti-CXCR5-BV605, Biolegend, Cat. #145513, clone L138D7), however no defect was observed in germinal center responses or anti-viral antibody titers in the serum of T-ChAT^{KO} mice (Fig. 10C, 10D) that could drive the higher viral titers in these experiments.

Example 5: ChAT expression in T cells enhances their migration and cytolytic activity in tissues of LCMV-CI13-infected mice.

[0115] The ability of CD8⁺ T cells to target and kill infected cells is critical for viral control (Kagi et al., 1994), but the reported impact of ACh on cytolytic lymphocyte (CTL) function varies. While addition of ACh boosted CTL activity in mixed lymphocyte reactions (Zimring et al., Regulation of CD8⁺ cytolytic T lymphocyte differentiation by a cholinergic pathway. Journal of Neuroimmunology, 2005. 164; 66-75), loss of specific cholinergic receptors in vivo did not alter the response to acute viral infection (Vezys et al., Analysis of CD8⁺ T cell-mediated anti-viral responses in mice with targeted deletions of the M1 or M5 muscarinic cholinergic receptors. Life Sciences, 2007. 80; 2330-2333).

[0116] To elucidate whether T-ChAT influences CTL activity, *in-vivo* killing of LCMV peptide-pulsed target cells in ChAT^{WT} and T-ChAT^{KO} cohorts was evaluated at 8 days post-LCMV-CI13 infection. At this time point, numbers of virus-specific CD8⁺ T cells and inhibitory receptor expression levels are equivalent in ChAT^{WT} and T-ChAT^{KO} mice (Fig. 4, 11A, 11B). Therefore, any differences observed in CTL activity are likely to be driven by intrinsic differences in the CTLs themselves. Although no differences in the specific lysis of GP276- or NP396-pulsed target cells were observed in the spleens of ChAT^{WT} and T-ChAT^{KO} animals (Fig. 5A), T-ChAT^{KO} mice could not eliminate GP276- and NP396- pulsed target cells in the liver (Fig. 5B). Intriguingly, GP33-specific lysis was also decreased in T-ChAT^{KO} spleen, with a trend towards reduced target cell elimination in the liver (Fig. 5A, B). This impaired CTL activity was not driven by changes in inhibitory receptor expression in the liver (Fig. 11C, 11D).

[0117] To efficiently kill infected cells, CD8⁺ T cells migrate out of the circulation and into the infected tissue. Intravascular staining (Anderson et al., Intravascular staining for discrimination of vascular and tissue leukocytes. Nature protocols, 2014. 9; 209-222) was performed to determine what fractions of virus-specific CD8⁺ T cells were in various tissues versus the blood vessels in LCMV-CI13-infected ChAT^{WT} and T-ChAT^{KO} mice. On day 8, post-infection the numbers of virus-specific CD8⁺ T cells in the blood

vessels of the liver, kidney, and salivary gland were similar in infected ChAT^{WT} and T-ChAT^{KO} mice (Fig. 5C). Strikingly, the number of virus-specific CD8⁺ T cells that migrated into each of these tissues was reduced in T-ChAT^{KO} mice (Fig. 5D). A similar defect was observed in the ability of IL-21R^{-/-} T cells to migrate into liver tissue by 8 days post-infection (Fig. 11E, 11F), suggesting that IL-21-driven ChAT expression enhances the migration of T cells into tissues.

[0118] To test if ChAT expression facilitates migration in a cell-intrinsic manner, adoptive transfers of 1x10⁴ ChAT^{WT} or T-ChAT^{KO} P14 TCR transgenic T cells into congenic CD45.1 ChAT^{WT} or CD45.1 T-ChAT^{KO} recipients were performed to establish 4 groups: ChAT^{WT} P14 into ChAT^{WT} recipients (WT->WT); ChAT^{WT} P14 into T-ChAT^{KO} P14 into ChAT^{WT} recipients (KO->WT) (Fig. 5E; anti-CD45.2-FITC, Biolegend Cat. #109806, clone 104). The P14 transgenic TCR is specific for the LCMV D^b(GP33) epitope, ensuring that all donor T cells will respond to this epitope of LCMV. Recipients were infected with LCMV-CI13 at 3 days post-transfer and the accumulation of P14 cells in various organs was evaluated by intravascular staining at 30 days post-infection.

[0119] To determine if ChAT expression in T cells conferred an advantage for migration, a comparison was performed of the ratio of transferred P14 cells to the total number of D^b(GP33)⁺ CD8⁺ T cells in the spleen to the ratio in the resident population of various organs. If the P14 cells migrated as well as the endogenous D^b(GP33)⁺ cells, then they should be equally represented in both the tissue and the spleen, and this ratio should approximate 1. As expected, ratio of 1 was observed in the WT->WT and KO->KO cohorts, where there should not be any difference in the ability of P14 cells to migrate (Fig. 5F). In contrast, a greater accumulation of P14 cells in the tissues of the WT->KO group compared to their frequency in the spleen was consistently observed, resulting in ratios of greater than 1 (Fig. 5F). Thus, compared to ChAT^{KO} T cells present in the same host, ChAT^{WT} cells have an intrinsic advantage in migrating into infected tissues. Surprisingly, ChAT^{KO} P14 cells migrated just as well as the endogenous ChAT^{WT} D^b(GP33)⁺ cells in the KO->WT recipients (Fig. 5F). These recipients maintain an intact immune response dominated by ChAT^{WT} cells responding to all immunogenic LCMV epitopes. Thus, while ChAT-expression facilitates migration in a cell-intrinsic manner, if a sufficient number of ChAT-expressing cells is present, it is not necessary for every cell to express ChAT to achieve adequate tissue migration.

[0120] Next, an examination was done to determine whether cell-intrinsic ChAT expression influences the exhaustion or deletion of anti-viral T cells in these P14 transfer cohorts. As expected, the P14 cells in the WT->WT group were retained at the highest level (Fig. 11G). Interestingly, the P14 cells in both the WT->KO and KO->WT groups were not retained as well as in the WT->WT group, indicating that ChAT expression in T cells does not directly sustain responses during persistent infection (Fig. 11G). Alternatively, the loss of the P14 T cells in the KO->WT group could indicate that these T-ChAT^{KO} cells are less effective in competing with the endogenous ChAT^{WT} D^b(GP33) response. Importantly, inhibitory receptor expression was equivalent in the WT->KO and KO->WT groups, indicating that ChAT does not act in a cell-intrinsic manner to modulate exhaustion (Fig. 11H, 11I). Thus, ChAT likely facilitates viral control primarily by enhancing T cell migration into infected tissues.

[0121] *In vivo* CTL activity in the liver was impaired for two epitopes examined 8 days post-infection in T-ChAT^{KO} mice (Fig. 5B), despite equivalent expression of granzyme B and degranulation (Fig. 5G) by non-circulating CTLs. The fraction of liver-infiltrating D^b(GP276)-specific CD8 T cells expressing granzyme B was determined by intravascular staining. The fraction of liver-infiltrating CD8 T cells expressing CD107a and IFN γ after *in vitro* stimulation with GP276 was compared to the total number of liver-infiltrating D^b(GP276)+ cells to determine the percent of GP276-specific cells capable of degranulation. Mean $\pm$ S.E.M., composite of 2 experimental cohorts n=11-12.

[0122] An analysis schematic for P14 transfer experiments is shown in FIG. 5H. ChAT^{WT} P14 or ChAT^{KO} P14 T cells were transferred into either ChAT^{WT} or T-ChAT^{KO} recipient mice, which were subsequently infected with LCMV-C113. Vascular cells in different tissues were marked as in A 30 days post-infection. The D^b(GP33)-specific non-vascular cells were evaluated to determine the relative ratio of endogenous and P14 cells in the spleen and peripheral organs. The relative abundance of P14 cells in the organs was then compared to the spleen, to determine whether P14 cell migrated better, as well, or worse than the endogenous D^b(GP33)-specific cells.

Example 6: ChAT expression in T cells increases blood vessel diameter during infection

[0123] The migration of immune cells into tissues is facilitated when blood flow slows, a function of the vasodilation that is a hallmark of inflammation. Vasodilation is triggered

when ACh signals to endothelial cells to produce NO (Furchott and Zawadzki, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, 1980. 288; 373-376). ChAT⁺ T cells were evaluated to see if they could alter blood vessel diameter and promote extravasation. The average blood vessel diameter was measured in fixed liver sections from LCMV-Cl13-infected ChAT^{WT} and T-ChAT^{KO} mice and the results found this parameter to be significantly large in the wild type animals at 8 days post-infection (Fig. 12A, 12B). By evaluating large z-stacks obtained with 2-photon microscopy, it was confirmed that small sinusoidal blood vessels were narrower in the T-ChAT^{KO} liver at day 8 post-infection (data not shown). Among blood vessels smaller than 20µm in diameter, both the mean and median blood vessel diameter were reduced in T-ChAT^{KO} mice at 8 days post-infection compared to their ChAT^{WT} counterparts (Fig. 6A, B). No consistent decrease was found in small blood vessel diameter at 30 days post-infection (Fig. 12C, 12D), a point after which ChAT levels have decreased. (Fig. 1).

[0124] Because the above approaches measured both arteries and veins, whereas vascular resistance and blood flow rate are driven only by arterial pressure, arterial vasodilation was examined by filling the arteries of ChAT^{WT} and T-ChAT^{KO} mice with the radio-opaque silicone polymer Microfil. At 8 days post-infection, blood vessels were filled and mouse livers were imaged using a micro-CT scanner. The arterial tree in T-ChAT^{KO} liver was largely depleted of branches compared to the tree in ChAT^{WT} liver (Fig. 6C, 6D, (data not shown)). The limit of vessel detection of these scans is ~30µm in diameter, and it is suspected that the T-ChAT^{KO} images fall below this limit of detection earlier than ChAT^{WT} images. Equivalent branches of ChAT^{WT} vessels were larger than T-ChAT^{KO} vessels at each branch depth after branch 3 (Fig. 6E). Significantly, treatment of T-ChAT^{KO} mice with the vasodilator minoxidil hydrochloride (Cohn et al., Direct-acting vasodilators. *Journal of Clinical Hypertension*, 2011. 13; 690-692) (minoxidil hydrochloride, Abcam Cat #ab141670) largely restored vasodilation in the liver (data not shown), while treatment of wild type C57Bl6 mice with the NO synthase (NOS) inhibitor L-NAME (Peotta, et al., Cardiovascular neural reflexes in L-NAME-induced hypertension in mice. *Hypertension*, 2001. 38; 555-559) (L-NAME Sigma Cat. #N5751, 400µg in PBS per gram body weight) recapitulated the smaller, stunted arterial tree observed in T-ChAT^{KO} mice (Fig. 6C-E).

[0125] Blood vessel diameters in naïve ChAT^{WT} or T-ChAT^{KO} mice did not differ (Fig. 6F, Fig. 12E, 12F (data not shown)). The mean vessel diameter in livers of naïve mice

was compared to those in mice of the same genotype at day 8 post-infection. As expected, substantial vasodilation occurred in infected ChAT^{WT} livers, with blood vessel diameters reaching ~150% of ChAT^{WT} naïve samples (Fig. 6G). In contrast, infected T-ChAT^{KO} livers showed no measureable increase in vessel diameter as compared to naïve samples (Fig. 6G). Thus, most if not all, of the vasodilation induced by viral infection is driven by ChAT-expressing T cells.

Example 7: Short-term treatment with vasodilators restores viral control and immune function in T-ChAT^{KO} mice.

[0126] If induction of local vasodilation to enhance immune cell migration is the main mechanism by which ChAT in T cells promotes viral control, then administration of minoxidil, which largely restored vasodilation in T-ChAT^{KO} mice, should also restore anti-viral responses. To test this prediction, minoxidil was administered to LCMV-Cl13-infected ChAT^{WT} and T-ChAT^{KO} mice by daily oral gavage on day 6 to day 12 post-infection (Fig. 7A), the period of peak ChAT expression (Fig. 1). Control mice were gavaged with water. At 30 days post-infection, a striking decrease in serum viral titer was observed in both minoxidil-treated groups (Fig. 7B) that corresponded to an increase in numbers of virus-specific T cells in their livers and salivary glands (Fig. 7C, D).

[0127] Minoxidil treatment did not alter virus-specific T cell accumulation in the lung (Fig. 7E), however the recovered virus-specific T cells expressed fewer inhibitory receptors, as did the virus-specific cells recovered from the lung tissue (Fig. 7F-H). Treatment of IL-21R^{-/-} mice with minoxidil also reduced serum viral titers by 30 days post-infection, but not to the levels in treated wild type mice (Fig. 7). This result highlights the multi-faceted role IL-21 plays in anti-viral defense.

[0128] In contrast to minoxidil treatment, treatment of WT C57Bl6 mice with L-NAME between days 6 and 12 post-infection resulted in high viral titers that were sustained until day 30 post-infection (Fig. 7I, 7J). Virus-specific T cells in these animals exhibited enhanced exhaustion as measured by inhibitory receptor expression (Fig. 7K). Collectively, these results demonstrate that direct modulation of vasodilation, either enhancing or inhibiting, during the early stages of virus infection can alter the outcome of viral infection irrespective of Ach production by T cells.

Example 8: Treatment with vasodilators increases cell infiltrate in TChAT^{KO} mice

transplanted with B16 melanoma.

[0129] T-ChAT^{KO} mice transplanted with B16 melanoma were given minoxidil (systemically) and BPP5a (locally) alone or in combination with a PD-1 inhibitor. Control groups with no treatment and PD-1 alone treatment were also included in the study. Immune cell infiltrate per mg tumor tissue was calculated for innate cells (FIG. 13) and lymphocytes (FIG. 14).

Example 9: Loss of ChAT expression in T cells from T-ChAT^{KO} mice.

[0130] Total CD4⁺ or CD8⁺ T cells were isolated from pooled secondary lymphoid organs of ChAT^{WT} (black, n=8) or T-ChAT^{KO} (white, n=9) mice 8 days post-LCMV C113 infection (FIG. 15). Naïve CD44^{lo} GFP⁻ CD4⁺ and CD8⁺ cells were isolated by cell sorting from pooled secondary lymphoid organs of 5 ChAT-GFP mice, and used as controls for ChAT expression. Expression was determined by the $\Delta\Delta$ CT method, using RSP9 as a housekeeping gene and normalized to either naïve CD4⁺ (right) or CD8⁺ (left) cells (FIG 15).

Example 10: nAChR α 7^{-/-} mice transplanted with B16 melanoma have less tumor growth and mass than wild type

[0131] nAChR α 7^{WT}, nAChR α 7^{+/-}, and nAChR α 7^{-/-} mice were transplanted with B16 melanoma. Tumor area (mm²) was assessed at 5 timepoints within 20 days post-transfer (FIG. 16A). Tumor mass was measured on day 20 post-transfer (FIG. 16B). The statistical significance is >0.05 for one *, and >0.0001 for four ****. In the top panel the significance denotes a change from the wild type.

Materials and Methods

[0132] Mice and infections. Chat-GFP (B6.Cg-Tg(RP23-268L19-EGFP)2Mik/J), Chat^{fllox} (B6.129-Chat^{tm1Jrs}/J), CD4-Cre (Tg(Cd4-cre)1Cwi/BfluJ), CD45.1 (B6.SJL-Ptprc^aPepc^b/BoyJ), Il21^{-/-} (B6N.129-IL21^{tm1Kopf}/J) animals were purchased from Jackson Laboratories and maintained in fully accredited facilities at Princess Margaret Cancer Centre within the University Health Network (UHN). Acute infections were established in 6-8-week-old male and female mice by i.p. injection of 2×10⁵ PFU LCMV-Armstrong. For protracted infections, 6-8-week-old male or female mice were infected i.v. with 2×10⁶ PFU of LCMV-Clone 13. In Chat-GFP studies, cages were randomly

selected for either LCMV-Arm or LCMV-Clone 13 infection. For cohorts of *Chat*-WT and *T-Chat*-KO mice, littermates were infected with the same preparation of either LCMV-Arm or LCMV-Clone 13. Therefore, these studies were not randomized. All procedures with experimental mice were approved by the UHN Animal Care Committee. All animals examined were included in the analyses. Investigators were not blinded to groups.

[0133]Lymphocyte isolation and peptide stimulation. Spleens were disrupted to generate single cells suspensions using a 70 µm sterile filter, and erythrocytes were removed by lysis with 0.83% (w/v) NH₄Cl. Cells were resuspended in RPMI 1640 containing 10% FCS, 50 µM β-mercaptoethanol, 100 U/mL penicillin, and 100 µg/mL streptomycin (R10). For liver lymphocyte isolation, gall bladders were removed prior to liver isolation. Livers were then disrupted to generate single-cell suspensions using a 100 µm sterile filter. Liver suspensions were overlaid on an 80/40 discontinuous Percoll (GE Healthcare Life Sciences) gradient and centrifuged for 20 minutes at 20°C at 711xg. Lymphocytes were isolated from the 80/40 interface and thoroughly washed in R10 prior to either staining or in vitro stimulation. For stimulations, cells were either left unstimulated or activated with LCMV-derived peptide epitopes (1 µg/mL) for 5 hours in the presence of brefeldin A (Golgi Plug, BD Biosciences).

[0134]P14 in vitro activation. 1×10⁵ whole splenocytes from P14-*Chat*-GFP⁺ or P14-*Chat*-GFP⁻ mice were plated in 96 well round-bottom plates and either left unstimulated or were activated with 1 µg/mL GP33 peptide and various cytokines (IFNβ at 2500U/mL, IL-2 at 100U/mL (high dose) or 10U/mL (low dose), IL-7 at 50ng/mL, IL-15 at 50ng/mL, IL-21 at 30ng/mL, IL-6 at either 30ng/mL (high dose) or 10ng/mL (low dose), IL-10 at either 50ng/mL (high dose) or 20ng/mL (low dose)). After 2 days of activation at 37°C, activated wells were transferred into a 24-well flat-bottom plate and cytokines and peptide were replenished at the same concentration. On day 5 of activation, wells were stained for CD8α and activation markers, and *Chat*-GFP expression was evaluated by flow cytometry.

[0135]In vivo killing assays. Spleen and lymph node cells were isolated from CD45.1⁺ congenic animals. One half of the total isolate was labeled with 2.5 µM CFSE in PBS, and the other half of the isolate labeled with 0.1 µM CFSE in PBS for 9 minutes at 37°C. Reactions were quenched with heat-inactivated FCS, and cells were washed and transferred to a new tube. One half of the cells from each reaction were then separated and labeled with 2.5mM VCT in PBS for 9 minutes at 37°C, and reactions were quenched with FCS as before. This labeling generated four discernible populations by

flow cytometry (CFSE^{hi}VCT⁻, CFSE^{hi}VCT⁺, CFSE^{lo}VCT⁻, and CFSE^{lo}VCT⁺). Each discrete population was labeled with 1 µg/mL of either GP33, NP396, or GP276 peptide for 1 hour at 37°C. One population of control cells was incubated without peptide for 1 hour. Following peptide pulsing, cells were washed and then mixed 1:1:1:1 to form the target cell pool. Target cells were injected i.p. in *Chat*^{WT} or T-*Chat*^{KO} animals infected with LCMV-Cl13 7 days previously. As a control, target cells were also injected in *Chat*^{WT} and T-*Chat*^{KO} LCMV-naïve animals. Recipient mice were sacrificed 8 hours after transfer, and single cell suspensions of spleen and liver were stained for CD45.1 and samples collected on a FACS Fortessa flow cytometer. Ratio of target cells was determined by gating on CD45.1⁺ cells and evaluating the relative proportions of CFSE^{hi}VCT⁻, CFSE^{hi}VCT⁺, CFSE^{lo}VCT⁻, and CFSE^{lo}VCT⁺ cells. Specific lysis was determined using the following formulas: ratio of recovery = (percentage of non-pulsed controls/peptide pulsed controls). Percent specific lysis = 100 × (1 - ratio of recovery from naïve mice/ratio of recovery from infected mice). Killing was then normalized to the specific killing of the wild-type response to facilitate comparison between experiments. Briefly, the specific killing for each epitope of each sample was divided by the average specific killing for that epitope of the wild type, enabling visualization of the fold decrease in specific killing in T-*Chat*^{KO} livers.

[00204] Microfil visualization and quantification of arterial vessel diameter.

Animals were euthanized with CO₂ and the vena cava was clamped in the thoracic cavity. The portal vein was cut to allow blood drainage, and the mouse was perfused with heparinized PBS via the heart. Once blanching was observed in the liver, Microfil (FlowTech Inc., Carver, MA) was mixed with the curing agent at a concentration to allow small blood vessel filling (2 mL of yellow pigmented compound, 5 mL of diluent, 255 µL of curing agent). The Microfil was then perfused via the heart, and the hepatic artery observed for the presence of Microfil. When the branches of the hepatic artery appeared filled and bright yellow, and small amounts of Microfil observed to be exiting the portal vein, the perfusion was ended. The vena cava was then cut in the thoracic cavity below the clamp, as well as in the abdominal cavity to prevent Microfil from draining into the veins in the liver. Mice were wrapped in aluminum foil and allowed to cure for a minimum of 4 hours. After curing, livers were carefully dissected to keep the celiac branch of the aorta intact, and livers fixed overnight in formalin. After fixation, livers were mounted in sampling tubes in 1% agarose and imaged on the Bruker SkyScan 1272 by the experienced staff at the Mouse Imaging Centre, Hospital for Sick

Children, Toronto. For these scans, the x ray tube voltage was set to 80kV, with a 0.5mm Al filter. View angles were collected through 180° with 0.4° steps. Images were reconstructed at 16 µm isotropic resolution. The 3D reconstruction of the data was analyzed in IMARIS version 9.1 (Bitplane Inc., Concord, MA). Arterial vessel diameter was determined in IMARIS using the draw tool to measure average vessel diameter. At each branch depth, multiple measurements were taken of each blood vessel, as visualized in movies 1-6. **Movie 1.** Naïve *Chat^{MT}* liver arterial tree filled with microfil and imaged by micro-CT. Demonstration of arterial measurements depicted as well as 3D structure of the liver arterial tree. Representative of 2 mice. **Movie 2.** Naïve *T-Chat^{KO}* liver arterial tree filled with microfil and imaged by micro-CT. Demonstration of arterial measurements depicted as well as 3D structure of the liver arterial tree. Representative of 2 mice. **Movie 3.** *Chat^{MT}* liver arterial tree 8 days post-LCMV CI-13 infection filled with microfil and imaged by micro-CT. Demonstration of arterial measurements depicted as well as 3D structure of the liver arterial tree. Representative of 4 mice. **Movie 4.** *T-Chat^{KO}* liver arterial tree 8 days post-LCMV CI-13 infection filled with microfil and imaged by micro-CT. Demonstration of arterial measurements depicted as well as 3D structure of the liver arterial tree. Representative of 4 mice. **Movie 5.** Minoxidil treated (D6-8) *T-Chat^{KO}* liver arterial tree 8 days post-LCMV CI-13 infection filled with microfil and imaged by micro-CT. Demonstration of arterial measurements depicted as well as 3D structure of the liver arterial tree. Representative of 3 mice. **Movie 6.** L-NAME treated (D6-8) C57Bl/6 liver arterial tree 8 days post-LCMV CI-13 infection filled with microfil and imaged by micro-CT. Demonstration of arterial measurements depicted as well as 3D structure of the liver arterial tree. Representative of 3 mice.

[0136] These values were entered into Excel, so that the mean diameter of each individual vessel at each branch depth was obtained. These individual mean vessel diameters were entered into Prism GraphPad software to generate plots. The number of terminal branches per liver sample were counted manually.

[0137] Antibodies and Cellular Analysis. Cell suspensions were stained with combinations of PECy7-labeled anti-PD-1 (clone RMP1-30), PE-labeled anti-Tim3 (clone RMT3-23), PerCP-Cy5.5-labeled anti-LAG3 (clone C9B7W), PE-Cy7-labeled anti-CD4 (clone RM4-5), Alexa700 labeled anti-CD44 (clone IM7) (all from Biolegend), eFlour450-labeled anti-CD8 (clone 53-6.7, eBioscience) and either the D^b(GP33) tetramer or the D^b(GP276) tetramer conjugated with streptavidin APC (NIH tetramer core facility). For Ki67 staining, cells were fixed after tetramer staining utilizing the

Cytofix/Cytoperm kit from BD, and FITC-labeled anti-Ki67 (clone SolA15, eBioscience) was stained intracellularly. Transcription factor staining was achieved using the FoxP3/Transcription Factor staining buffer kit from Affymetrix (formerly eBioscience) to fix and permeabilize cells. PerCP-Cy5.5-labeled anti-Tbet (clone 4B10) and PE-labeled anti-IRF4 (clone IRF4.3E4) (Biolegend) were stained intracellularly. For intracellular cytokine staining, stimulated samples were stained with anti-CD8 (clone 53-6.7), then permeabilized with the Cytofix/Cytoperm kit from BD and stained intracellularly for IFN γ (clone XMG1.2), TNF α (clone MP6-XT22), and IL-2 (clone JES6-5H4). Samples were acquired on either a FACS-Canto II or a Fortessa flow cytometer (BD), and data analyzed by using FlowJo software (Tree Star).

[0138] Intravascular staining. Staining of cells in the circulation was performed as described (15). Briefly, animals were injected with 3 μ g of FITC-labeled α -CD8 antibody (clone CT-CD8a) i.v. and sacrificed 3 minutes after injection. Blood and lymph nodes were isolated as positive and negative controls, respectively. Lymphocytes isolated from blood, lymph nodes, spleen, and liver were stained with eFlour450-labeled anti-CD8 (clone 53-6.7, eBioscience) and APC-labeled tetramer (NIH tetramer core facility) to evaluate the number of virus-specific CD8 T cells that had migrated into the liver tissue (FITC⁻) versus the number of cells in the circulation (FITC⁺).

[0139] Determination of viral titer. Blood was collected in a BD Microtainer SST chemistry tube (BD Biosciences), allowed to clot, and spun in a refrigerated microfuge at 28,000g for 2 minutes at 4 degrees Celsius. Serum was serially diluted and PFU determined using Vero cell monolayers.

[0140] PCR analysis for *Chat* expression. Pooled splenocytes from a minimum of five mice were purified for CD4⁺ and CD8⁺ T cells using the Invitrogen Dynabeads FlowComp CD8 and CD4 kit (ThermoFisher Scientific). For GFP⁺ samples, cells were further sorted based on *Chat*-GFP, CD4, CD8, and, for naïve T cells, CD44^{lo} using the BD Aria cell sorter (BD Biosciences, Franklin Lakes, NJ). Frozen cell pellets were prepared from isolated populations. RNA was isolated from cell pellets using the Nucleospin RNA Plus kit (Macherey-Nagel, Bethlehem PA) and cDNA generated using the iScript cDNA synthesis kit (BioRad). RT-PCR was performed using Power SyberGreen PCR Master Mix (ThermoFisher Scientific). Primer sequences as follows: *mChat* fwd - 5' - CCATTGTGAAGCGGTTTGGG - 3'; *mChat* rev - 5' - GCCAGGCGGTTGTTTAGATACA - 3'; *mRSP9* fwd - 5' - CAAGATGAAGCTGGATTAC - 3'; *mRSP9* rev - 5' - GGGATGTTCCACCACCTG - 3'

[0141] Minoxidil and L-NAME Treatment. Infected mice were weighed 6 days post-infection to find a body weight, and for minoxidil treatment gavaged daily through day 12 post-infection with either 2mg/kg minoxidil hydrochloride or an equivalent volume of MilliQ water. In L-NAME treated cohorts, mice were injected with either 400µg/g of L-NAME dissolved in PBS i.p. or an equivalent volume of PBS daily beginning at day 6 through day 12 post-infection. For evaluation of blood vessel diameter on day 8, mice were treated a final time on day 8 prior to euthanasia and analysis.

[0142] Detection of Acetylcholine by Mass Spectrometry. A 48 well plate was coated overnight with either 1µg/mL anti-mouse CD3e (BD Biosciences) in PBS or PBS alone. *Chat*^{WT} and T-*Chat*^{KO} mice were infected with LCMV-Armstrong and CD8⁺ CD44^{hi} effector cells were sorted 8 days post-infection. After sorting cells were washed and resuspended in PBS. Cells were rested in PBS at 37°C for 1 hour, and then plated in either control or anti-CD3-coated wells. Cells were stimulated at 37° for 15 minutes, and then the cell suspension was transferred into 1.5mL Eppendorf tubes and spun at 28,000g for 10 minutes at 4°C. 100µL of the supernatant was then removed and mixed with 100µL of methanol. Liquid chromatography-mass spectrometry (LC-MS) analysis of Acetylcholine was performed on these supernatants using a Dionex Ultimate 3000 UHPLC system and a Q-Exactive mass spectrometer equipped with a HESI II source (all from Thermo Scientific) and controlled by Thermo XCalibur 4.1 software. LC separation was conducted on a Luna NH2 column (150 mm × 2 mm, 3 µ particle size, Phenomenex) equipped with a guard column. Solvent A was 0.1% formic acid in water, solvent B was 0.1% formic acid in acetonitrile (flow rate 0.3 ml/min). Autosampler temperature was maintained at 10°C, and injection volume was 15 µl. The gradient was 0 – 1 min: 2% B; 1 – 7 min: 2%-98% B; 7 – 10.0 min: 98% B; 10 – 10.5 min: 98%B - 2% B; 10.5 – 15 min: 2% B. Data collection was done in positive ionization mode with a scan range m/z 100–500, resolution 140000 at 1 Hz, AGC target of 3e6 and a maximum injection time of 250 ms. Standard solutions of Acetylcholine (m/z 146.1176) were used for validation of retention time and m/z.

[0143] Statistical Analysis. Statistical significance between two populations was determined by using a two-tailed unpaired *t* test (Excel, Microsoft). F-tests were also performed, and where samples demonstrated unequal variation, a two-tailed *t* test assuming unequal variance was used to evaluate significant differences between populations. Statistical significance between more than two populations was analyzed by either one-way ANOVA or two-way ANOVA in Prism (GraphPad Software Inc, La

Jolla, CA. USA), and if significance was found samples were further analyzed pair-wise for significant difference by *t* test.

Results and Discussion

[00205] In this study, it was determined that *Chat* is induced by IL 21 in T cells during infection to facilitate T-cell entry into infected tissues, thereby genetically identifying the function of T-cell-derived ACh during an immune response.

[00206] *Chat*⁺ CD4⁺ T cells uniformly exhibit an “antigen-experienced” phenotype (4). Yet, the signals that drive *Chat* expression in T cells are undefined. *Chat*-GFP reporter mice (7) were infected with the rapidly cleared Armstrong strain of lymphocytic choriomeningitis virus (LCMV-Arm). There was a massive increase in *Chat*-GFP expression in both CD4⁺ and CD8⁺ T cells 8 days post-infection (Fig. 17A, B). In splenic virus-specific T cells, expression rapidly declined following LCMV-Arm clearance, yet *Chat*-GFP expression was retained in both virus-specific CD4⁺ and CD8⁺ T cells from mice chronically infected with LCMV clone-13 (LCMV-CI13) (Fig. 17C, D). GFP expression correlated with *Chat* mRNA in T cells (Fig. 1L). In CD4⁺ T cells, *Chat*-GFP was expressed by all subsets, however expression was highest in T follicular helper (T_{fh}) cells (Fig. 2A-D). In CD8⁺ T cells, there was no correlation with either memory precursor or short-lived effector phenotypes (Fig. 2E). Furthermore, *Chat*-GFP was induced in germinal center (GC) B cells in the spleen, although *Chat* expression was not retained in this population during persistent infection (Fig. 1I, J). *Chat*-GFP was also induced in both CD4⁺ and CD8⁺ T cells following vesicular stomatitis virus (VSV) infection (Fig. 18A, B).

[00207] The kinetics of *Chat*-GFP expression during acute and chronic infection implicates viral signals in driving *Chat* induction in T-cells. Viral infection induces numerous cytokines that influence T-cells, including type I interferons (IFN-I), IL-2, IL-6, IL-7, IL-10, IL-15, and IL-21 (8). *Chat*-GFP P14 TCR transgenic T cells were activated in vitro with the GP33 peptide in the presence or absence of these cytokines. Markedly, the only condition that resulted in *Chat* induction in P14 cells in vitro was IL-21 with peptide stimulation (Fig. 3A, 8A). The contribution of IL-21 signaling to *Chat* induction was evaluated in vivo by infecting IL-21 receptor-deficient (*Il21r*^{-/-}) (9) mice expressing the *Chat*-GFP reporter with LCMV-CI13. A decrease in the fraction of both CD4⁺ and CD8⁺ T cells expressing *Chat*-GFP in *Il21r*^{-/-} mice was observed (Fig. 3B). Mice heterozygous for *Il21r* (*Il21r*^{+/-}) showed a mixed phenotype. The expression of *Chat*-GFP in B-cell populations was not reduced in *Il21r*^{-/-} animals (Fig. 3B). *Chat*-GFP⁺ cells

in *Il21r^{-/-}* mice also demonstrated a lower mean fluorescence intensity (MFI) for the reporter molecule, suggesting reduced expression (Fig. 3D, 8B).

[00208] IL-21 is critical for anti-viral immunity (10-12). Thus, the role of IL-21-induced T-*Chat* was investigated by utilizing *Chat^{flox}* mice (13) crossed with CD4-cre mice (14) to generate *Chat^{flox/flox}* CD4-cre⁻ (*Chat^{WT}*) and *Chat^{flox/flox}* CD4-cre⁺ (T-*Chat^{KO}*) animals. Cre-driven recombination occurs at the double-positive stage in the thymus (14), resulting in deletion of *Chat* in both CD4⁺ and CD8⁺ T cells (Fig. 15A), and a subsequent failure to produce ACh (Fig. 15B). Strikingly, the loss of *Chat* specifically within T cells resulted in a failure to control LCMV-Cl13 in a subset of the animals (Fig. 4I), revealing that *Chat* expression in T cells is required during chronic infection. This failure to control LCMV-Cl13 corresponded with the attrition of virus-specific CD8⁺ T cells over time (Fig. 4A), poor cytokine production (Fig. 4F), and increased expression of inhibitory receptors (Fig. 19A, B). There was no difference in the numbers of antiviral T cells in LCMV-Arm infected T-*Chat^{KO}* mice (Fig. 22), which has also been reported for *Il21^{-/-}* animals (15). Although high *Chat* expression in T_{fh} and GC B cells was observed, there were no deficits in either anti-viral CD4⁺ T cell numbers or in the anti-LCMV antibody response in T-*Chat^{KO}* mice (Fig. 10).

[00209] Loss of IL-21 signaling results in decreased T-cell infiltration of tissues in bone marrow chimeras (16, 17). Thus, tissue-infiltration by *Il21r^{-/-}* T cells during LCMV-Cl-13 infection was evaluated using intravascular staining (18). A reduction in virus-specific T cells that had migrated into infected livers of *Il21r^{-/-}* mice was found (Fig. 20A). *Chat*-expressing T cells reduce blood pressure by producing ACh (3, 6), which may facilitate T-cell entry into tissues by slowing blood flow. Consequently, a reduction in virus-specific CD8⁺ T cells was also found in both the liver and salivary gland of T-*Chat^{KO}* mice after LCMV-Cl13 (Fig. 20B, C). Significantly, no difference in the number of circulating virus-specific cells was found in either *Il21r^{-/-}* or T-*Chat^{KO}* mice (Fig. 23A-C). A similar trend was observed in the liver for virus-specific CD4⁺ T cells (Fig. 23D). Poor migration into tissues could impact viral control, as fewer migrated cytotoxic T lymphocytes (CTLs) would result in the poor elimination of infected cells. In vivo CTL activity in the liver was impaired for two epitopes examined 8 days post-infection in T-*Chat^{KO}* mice (Fig. 20D), despite equivalent expression of granzyme B and degranulation (Fig. 5G) by non-circulating CTLs. Furthermore, this diminution in CTL activity was only observed for the GP33 epitope in the spleen (Fig. 20D), while not

wishing to be limited by theory, suggesting that the poor CTL activity in the liver was not due to intrinsic defects in the cells, but rather their impaired infiltration of tissues.

[00210] It was tested whether *Chat* expression in T cells functions in a cell-intrinsic manner by transplanting *Chat*^{WT} or T-*Chat*^{KO} TCR transgenic P14 T cells into congenic recipient *Chat*^{WT} or T-*Chat*^{KO} mice and infecting with LCMV-Cl13. The migration capacity of the P14 cells was then quantified (Fig. 5H). Briefly, the total non-circulating D^b(GP33)⁺ in the spleen and organs was examined and it was determined what percentage of these D^b(GP33)⁺ cells were donor P14 cells. This percentage in each organ was then directly compared to the spleen of that individual animal, to determine whether the P14 cells had migrated better (ratio>1), worse (ratio<1), or equivalently (ratio=1) to the endogenous D^b(GP33)⁺ T cells (Fig. 5H). In control mice (WT→WT and KO→KO), the frequency of P14 cells was similar in the spleen and organs, resulting in a ratio of approximately 1 (Fig. 5F, 23G). However, in T-*Chat*^{KO} mice receiving *Chat*^{WT} P14 cells, a greater frequency of *Chat*^{WT} P14 cells in the liver and kidney was observed than would be predicted by their rate in the spleen (Fig. 5F, 23G). Thus, *Chat*^{WT} cells were more efficient at seeding these peripheral organs than T-*Chat*^{KO} cells in the same animal. When T-*Chat*^{KO} P14 cells were transplanted into a *Chat*^{WT} recipient, they migrated as well as endogenous *Chat*^{WT} cells, indicating the observed differences were not due to an intrinsic defect of T-*Chat*^{KO} cells to adhere or sense chemokines. While not wishing to be limited by theory, this migratory advantage of *Chat*^{WT} cells in a T-*Chat*^{KO} host may be due to local changes in the vasculature induced by the presence of *Chat*⁺ T cells, and would still occur in *Chat*^{WT} recipients of T-*Chat*^{KO} P14 cells.

[00211] Vasodilation is critical for immune responses, and is one of the hallmarks of inflammation facilitating the entry of immune cells into infected tissues. Not only do *Il21r*^{-/-} mice exhibit smaller arterial connections in the brain (19), T-*Chat*^{KO} mice exhibit higher blood pressure than *Chat*^{WT} littermates (6), indicating they also have smaller arteries. ACh signaling has long been known to induce vasodilation (20). While not wishing to be limited by theory, *Chat*⁺ T cells induced by infection may be the primary mediators of vasodilation via the release of ACh, and that loss of *Chat* in T cells would consequently abrogate infection-driven vasodilation. Upon imaging the liver arterial vasculature of naïve and LCMV-Cl13 infected *Chat*^{WT} and T-*Chat*^{KO} mice (movies 1-4), it was found that infection-induced vasodilation in the liver was completely abrogated in T-*Chat*^{KO} mice, in contrast to their *Chat*^{WT} counterparts (Fig. 6C,F and G), resulting in

fewer detectable terminal branches (Fig. 6D) and smaller mean vessel diameter at equivalent branch depths (Fig. 6E).

[00212] The blood vessel phenotype in T-*Chat*^{KO} mice was reversed with short-term treatment with the vasodilator minoxidil (Fig. 6C-E, movie 5). Thus, these differences were not developmental, but reflective of poor vasodilation in the absence of T-*Chat*. Furthermore, treatment of wild-type mice with the vasoconstrictor L-NAME was sufficient to recapitulate the vascular phenotype observed in T-*Chat*^{KO} mice (Fig. 6C-E, movie 6). Minoxidil treatment was sufficient to restore viral control in T-*Chat*^{KO} mice, and also augmented viral control in *Chat*^{WT} animals (Fig. 21B). Moreover, viral titers were significantly higher in wild-type mice treated with L-NAME on days 6-12 post-infection compared to PBS-treated controls (Fig. 21C). Thus, vasodilation mediated by *Chat*-expressing T cells is critical for appropriate viral control.

[00213] IL-21 supports antiviral immunity beyond *Chat* induction and vasomodulation (21). Indeed, treatment with minoxidil was not sufficient to fully rescue *IL21*^{-/-}, although this treatment did reduce viral titers compared to vehicle-treated *IL21*^{-/-} mice. Efficient migration of effector T cells into tissues is critical for the control of viral infections (22), and is also of great interest for immunotherapy directed at tumors (23). In addition to its other reported roles during infection, IL-21 signaling enhances the efficacy of expanded tumor infiltrating lymphocytes to combat cancer (24, 25). Here, it is reported that IL-21, a cytokine critical for control of chronic infection (10-12), drives the expression of *Chat* in T cells to facilitate their migration into infected tissues. These findings underscore the role for IL-21 during the host response to infection and establish a cholinergic mechanism for regulating cellular migration into tissues.

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CLAIMS

What is claimed:

1. A method for treatment of cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition modulating choline acetyltransferase in T cells.
2. The method of claim 1, wherein the modulating comprises increasing expression of choline acetyltransferase.
3. The method of claim 1, wherein the modulating comprises decreasing expression of choline acetyltransferase.
4. The method of claim 1, wherein the pharmaceutical composition modulating choline acetyltransferase in T cells comprises IL-21.
5. The method of claim 1, wherein the pharmaceutical composition modulating choline acetyltransferase in T cells comprises TGF- β .
6. The method of claim 1, wherein the modulating comprises increasing activity of choline acetyltransferase.
7. The method of claim 1, wherein the modulating comprises decreasing activity of choline acetyltransferase.
8. The method of any one of claims 1-7, further comprising administering one or more anti-cancer therapeutic agents.
9. The method of claim 8, wherein the one or more anti-cancer therapeutic agents comprises at least one checkpoint inhibitor.
10. The method of claim 9, wherein the at least one checkpoint inhibitor is a member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.

11. The method of any one of claims 8-10, wherein the pharmaceutical composition modulating choline acetyltransferase in T cells and the one or more anti-cancer therapeutic agents are administered locally.
12. The method of any one of claims 8-10, wherein the pharmaceutical composition modulating choline acetyltransferase in T cells and the one or more anti-cancer therapeutic agents are administered systemically.
13. The method of any one of claims 8-10, wherein the pharmaceutical compound modulating choline acetyltransferase in T cells is administered locally and one or more of the one or more anti-cancer therapeutic agents is/are administered systemically.
14. The method of any one of claims 8-10, wherein the pharmaceutical compound modulating choline acetyltransferase in T cells is administered systemically and one or more of the one or more anti-cancer therapeutic agents is/are administered locally.
15. The method of any one of claims 8-14, wherein the pharmaceutical composition is conjugated to at least one of the one or more anti-cancer therapeutic agents.
16. A method for treatment of cancer to a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of T cells engineered to increase expression of choline acetyltransferase.
17. The method of claim 16, wherein the T cells are autologous.
18. The method of claim 16, wherein the T cells are antigen-specific.
19. The method of any one of claims 16-18, further comprising administering one or more anti-cancer therapeutic agents.
20. The method of claim 19, wherein the one or more anti-cancer therapeutic agents comprises at least one checkpoint inhibitor.

21. The method of claim 20, wherein the at least one checkpoint inhibitor is a member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.
22. A method for treatment of cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of a virus that is engineered to mediate induction of choline acetyltransferase.
23. The method of claim 22, further comprising administering one or more anti-cancer therapeutic agents.
24. The method of claim 23, wherein the one or more anti-cancer therapeutic agents comprises at least one checkpoint inhibitor.
25. The method of claim 24, wherein the at least one checkpoint inhibitor is a member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.
26. A method for the treatment of cancer in a subject in need thereof, the method comprising
- (a) obtaining a sample from the subject;
 - (b) measuring the level of choline acetyltransferase present in the sample;
 - (c) assessing that the subject has low choline acetyltransferase expression; and
 - (d) administering a pharmaceutically effective amount of a vasodilator.
27. The method of claim 26, the method comprising measuring the level of choline acetyltransferase present in the sample by immunoassay.
28. The method of claim 26, the method further comprising measuring the level of choline acetyltransferase present in the sample by a polymerase chain reaction assay.
29. The method of any one of claims 26-28, further comprising administering one or more anti-cancer therapeutic agents with the vasodilator.

30. The method of claim 29, wherein the one or more anti-cancer therapeutic agents comprises at least one checkpoint inhibitor.

31. The method of claim 30, wherein the at least one checkpoint inhibitor is a member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.

32. The method of any one of claims 29-31, wherein the vasodilator and the one or more anti-cancer therapeutics is/are delivered locally.

33. The method of any one of claims 29-31, wherein the vasodilator and the one or more anti-cancer therapeutics is/are delivered systemically.

34. The method of any one of claims 29-31, wherein the vasodilator is delivered locally and one or more of the one or more anti-cancer therapeutics is/are delivered systemically.

35. The method of any one of claims 29-31, wherein the vasodilator is delivered systemically and one or more of the one or more anti-cancer therapeutics is/are delivered locally.

36. The method of any one of claims 29-31, wherein the vasodilator is conjugated to at least one of the one or more anti-cancer therapeutic agents.

37. The method of any one of claims 26-36, wherein the vasodilator is coated on T cells *ex-vivo* and the T cells coated by the vasodilator are administered to the subject.

38. The method of any one of claims 26-37, wherein the vasodilator increases tumor infiltrating lymphocytes in a tumor.

39. A method for the treatment of cancer in a subject in need thereof, the method comprising:

- (a) obtaining a sample from the subject;
- (b) measuring the activity of choline acetyltransferase present in the sample;

- (c) assessing that the subject has low choline acetyltransferase activity; and
- (d) administering a pharmaceutically effective amount of a vasodilator.

40. The method of claim 39, wherein measuring the activity of choline acetyltransferase present in the sample is determined by measuring relative levels of a member selected from the group consisting of acetylcholine, choline, and acetyl-CoA.

41. The method of claim 39 or 40, the method comprising measuring the level of choline acetyltransferase present in the sample by immunoassay or enzyme assay.

42. The method of any one of claims 39-41, further comprising administering one or more anti-cancer therapeutic agents with the vasodilator.

43. The method of claim 42, wherein the one or more anti-cancer therapeutic agents comprises at least one checkpoint inhibitor.

44. The method of claim 43, wherein the at least one checkpoint inhibitor is a member selected from the group consisting of a PD-1 inhibitor, a PD-L1 inhibitor, a CTLA-4 inhibitor, a TIM-3 inhibitor, and a LAG-3 inhibitor.

45. The method of any one of claims 42-44, wherein the vasodilator and the one or more anti-cancer therapeutics is/are delivered locally.

46. The method of any one of claims 42-44, wherein the vasodilator and the one or more anti-cancer therapeutics is/are delivered systemically.

47. The method of any one of claims 42-44, wherein the vasodilator is delivered locally and one or more of the one or more anti-cancer therapeutics is/are delivered systemically.

48. The method of any one of claims 42-44, wherein the vasodilator is delivered systemically and one or more of the one or more anti-cancer therapeutics is/are delivered locally.

49. The method of any one of claims 42-48, wherein the vasodilator is conjugated to at least one of the one or more anti-cancer therapeutic agents.

50. The method of any one of claims 39-49, wherein the vasodilator is coated on T cells *ex-vivo* and the T cells coated by the vasodilator are administered to the subject.

51. A method for treatment of cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective pharmaceutical composition comprising of choline acetyltransferase.

52. The method of any one of the previous claims, wherein the cancer is melanoma.

53. The method of any one of the previous claims, wherein the method or pharmaceutical composition increases tumor infiltrating lymphocytes in a tumor.

54. A method for the treatment of viral infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition modulating choline acetyltransferase in T cells.

55. The method of claim 54, wherein the modulating comprises increasing expression of choline acetyltransferase.

56. The method of claim 54, wherein the modulating comprises decreasing expression of choline acetyltransferase.

57. The method of claim 54, wherein the pharmaceutical composition modulating choline acetyltransferase in T cells comprises IL-21.

58. The method of claim 54, wherein the pharmaceutical composition modulating choline acetyltransferase in T cells comprises TGF- β .

59. The method of claim 54, wherein modulating comprises increasing activity of choline acetyltransferase.

60. The method of claim 54, wherein modulating comprises decreasing activity of choline acetyltransferase.

61. The method of any one of claims 54-60, further comprising administering one or more anti-viral therapeutic agents.

62. The method of claim 61, wherein the pharmaceutical composition is conjugated to at least one of the one or more anti-viral therapeutic agents

63. A method for the treatment of viral infection in a subject in need thereof, the method comprising

- (a) obtaining a sample from the subject;
- (b) measuring the level of choline acetyltransferase present in the sample;
- (c) assessing that the subject has low choline acetyltransferase expression; and
- (d) administering a pharmaceutically effective amount of a vasodilator.

64. A method of claim 63, the method comprising measuring the level of choline acetyltransferase present in the sample by immunoassay.

65. A method of claim 63, the method further comprising measuring the level of choline acetyltransferase present in the sample by a polymerase chain reaction assay.

66. The method of any one of claims 63-65, further comprising administering one or more anti-viral therapeutic agents.

67. The method of claim 66, wherein the vasodilator is conjugated to at least one of the one or more anti-viral therapeutic agents.

68. A method for the treatment of viral infection in a subject in need thereof, the method comprising:

- (a) obtaining a sample from the subject;
- (b) measuring the activity of choline acetyltransferase present in the sample;
- (c) assessing that the subject has low choline acetyltransferase activity; and
- (d) administering a pharmaceutically effective amount of a vasodilator

69. The method of claim 68, wherein measuring the activity of choline acetyltransferase present in the sample is determined by measuring relative levels of a member selected from the group consisting of acetylcholine, choline, and acetyl-CoA.

70. The method of claim 68 or 69, the method comprising measuring the level of choline acetyltransferase present in the sample by immunoassay and/or polymerase chain reaction assay.

71. The method of any one of claims 68-70, further comprising administering one or more anti-viral therapeutic agents.

72. The method of claim 71, wherein the vasodilator is conjugated to at least one of the one or more anti-viral therapeutic agents.

73. A method for treatment of viral infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective pharmaceutical composition comprising choline acetyltransferase.

74. The method of any one of claims 54-73, wherein the method or pharmaceutical composition increases viral clearance.

FIG. 1

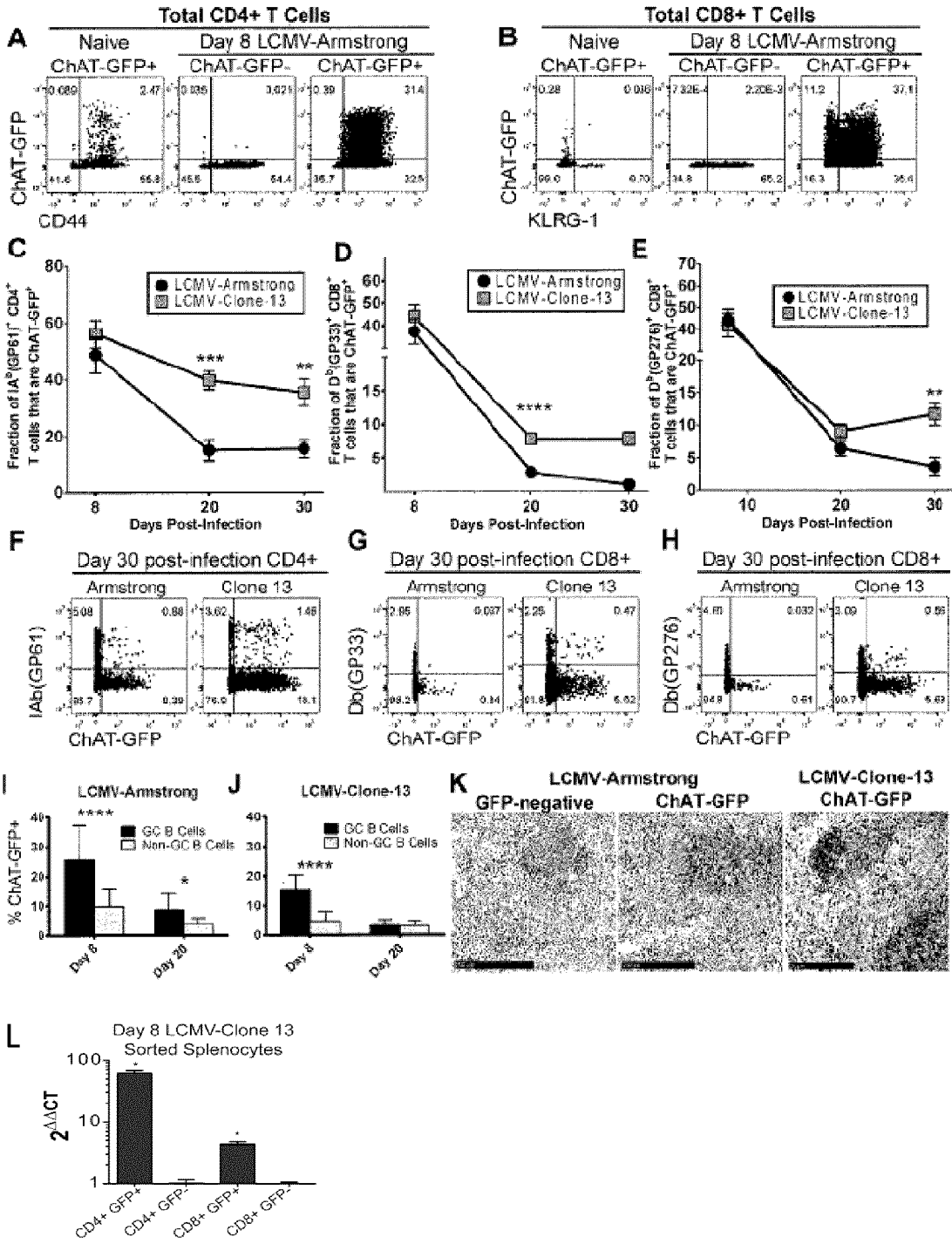


FIG. 2

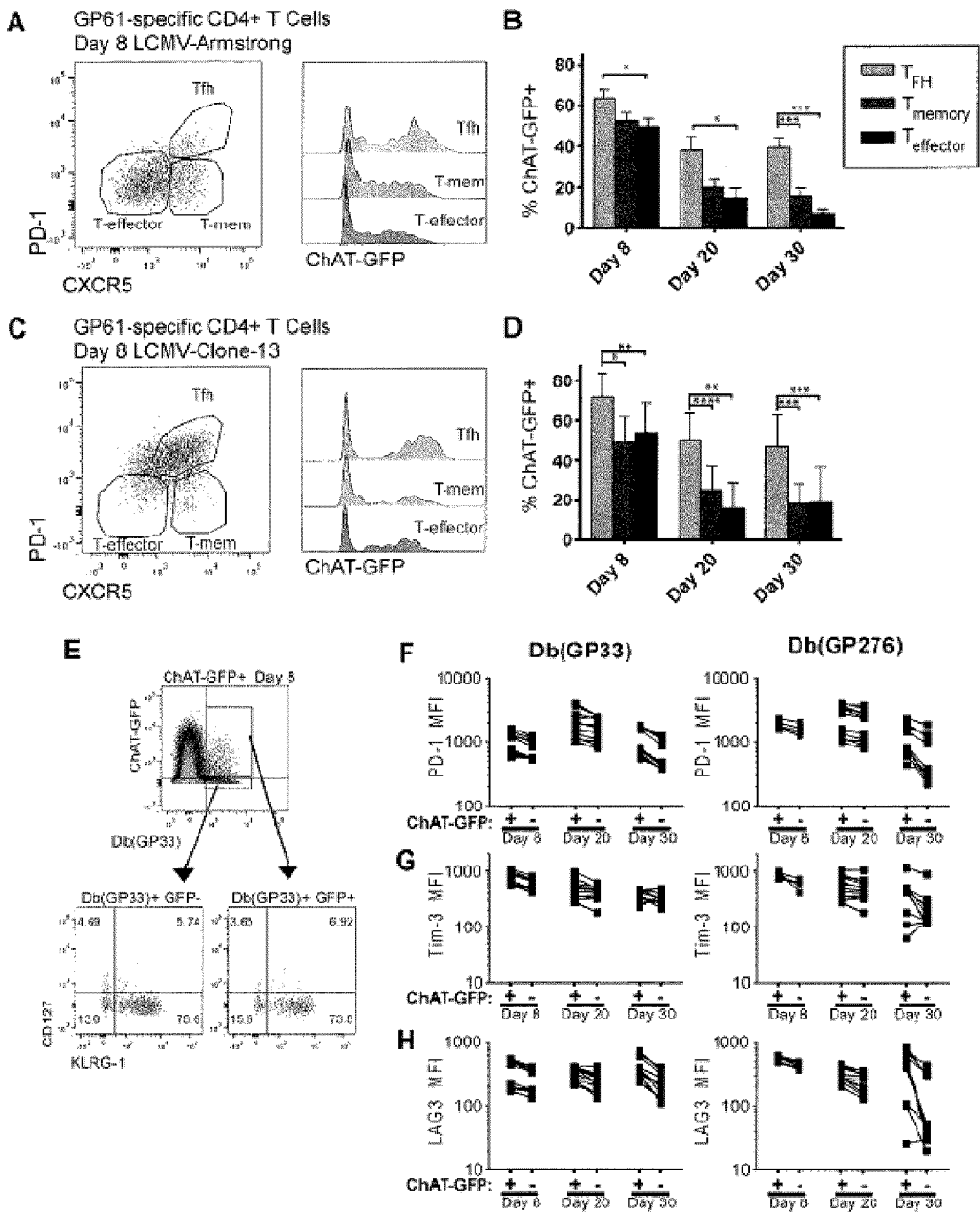


FIG. 3

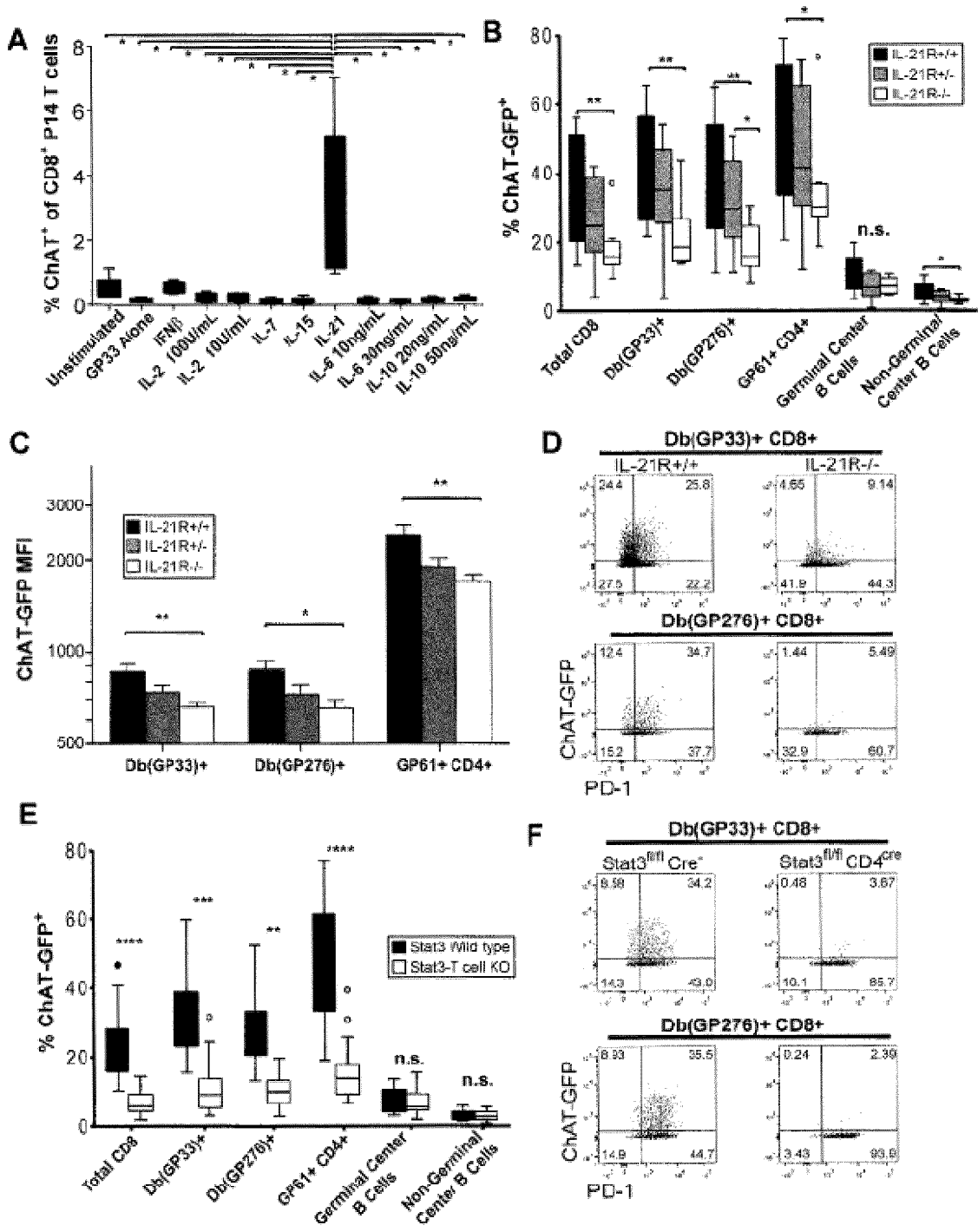
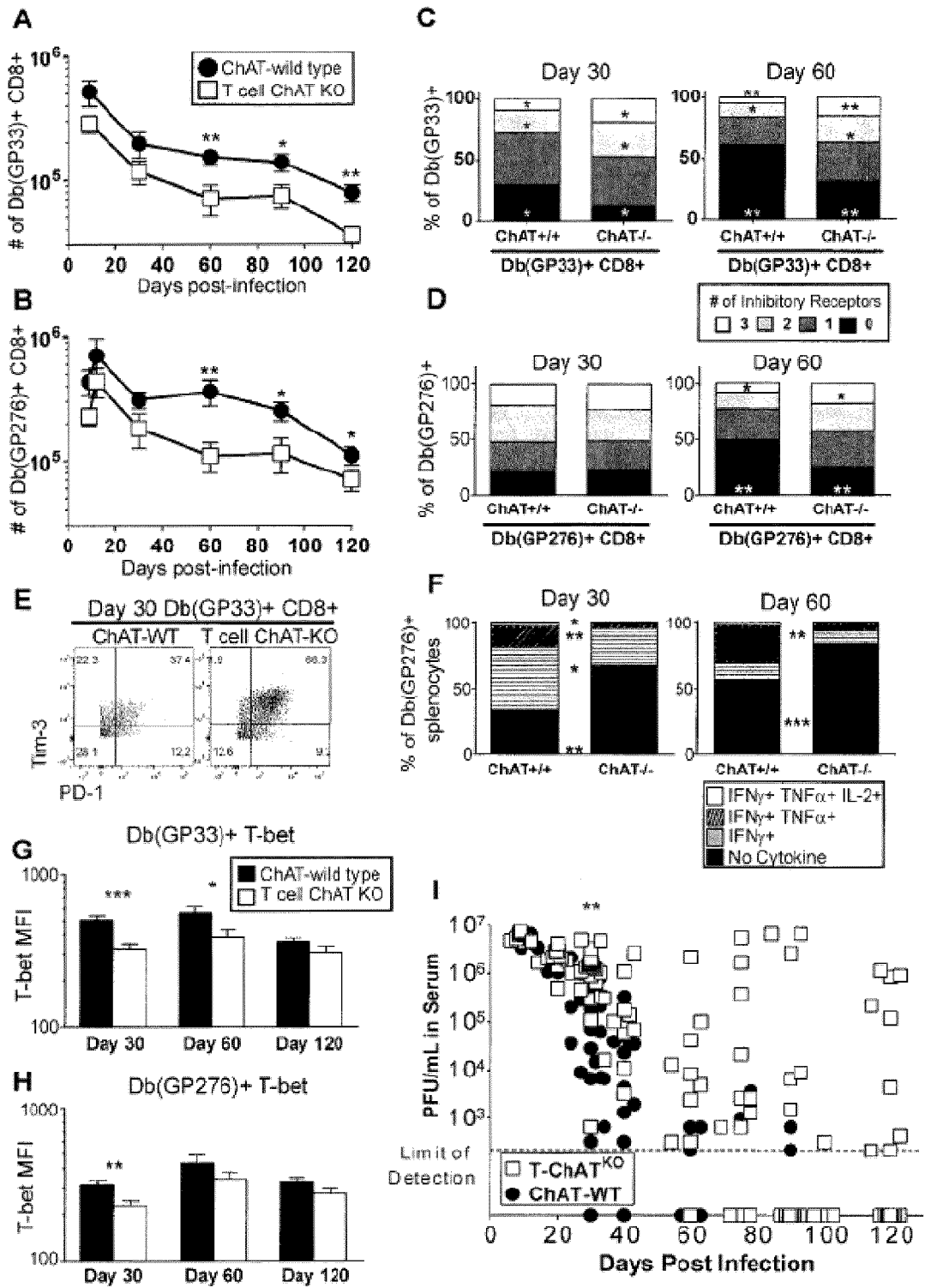
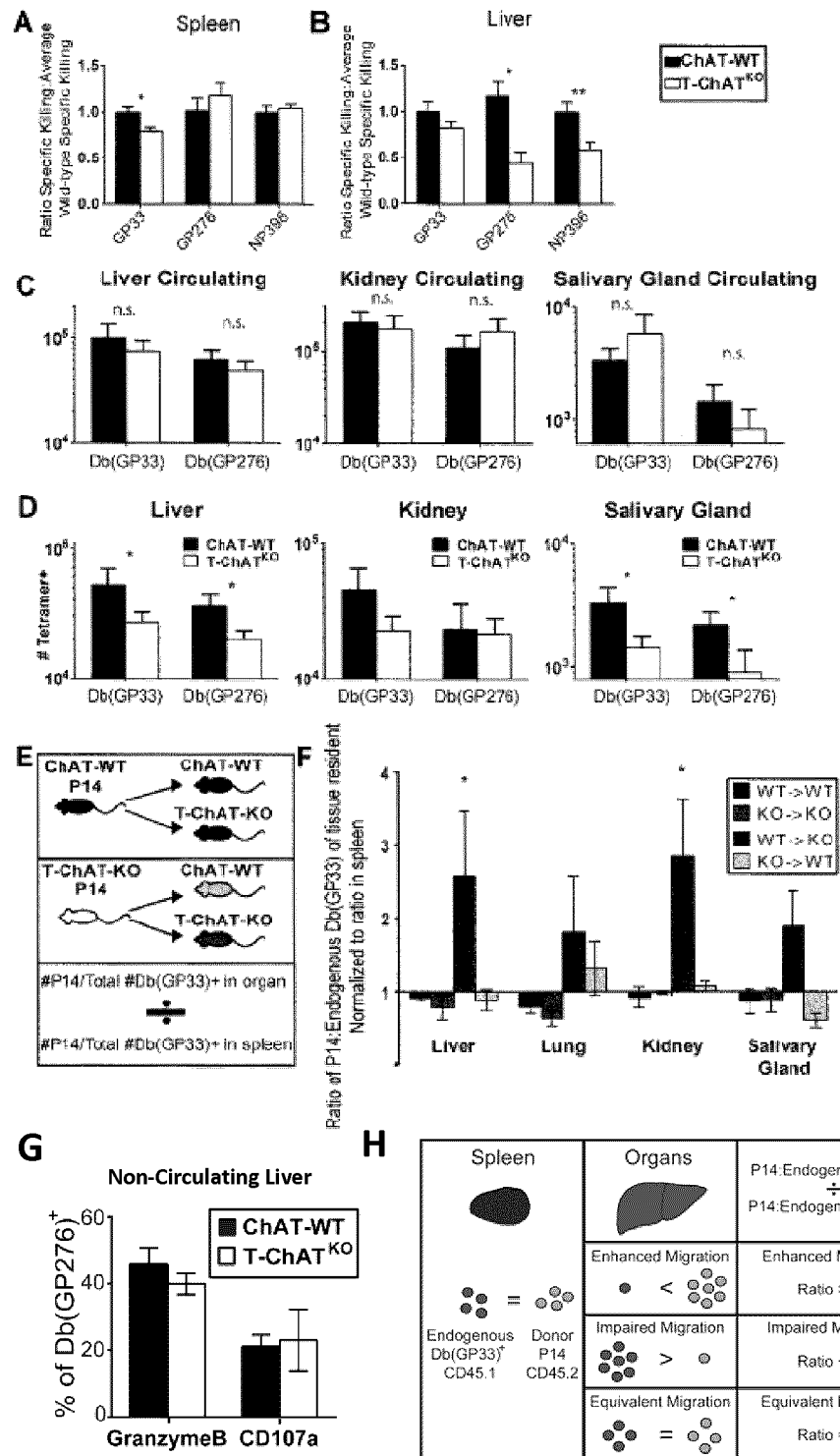


FIG. 4





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FIG. 6

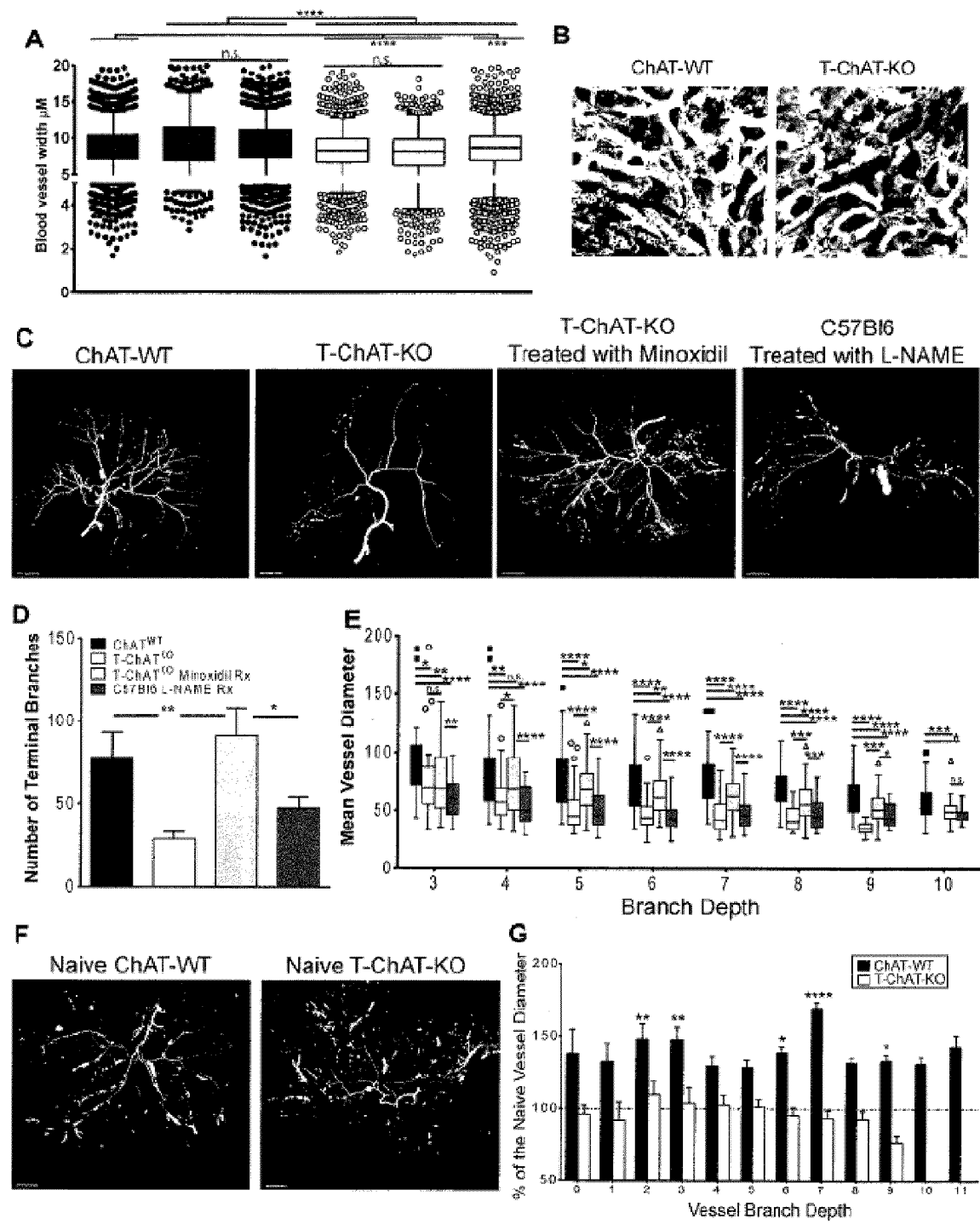


FIG. 7

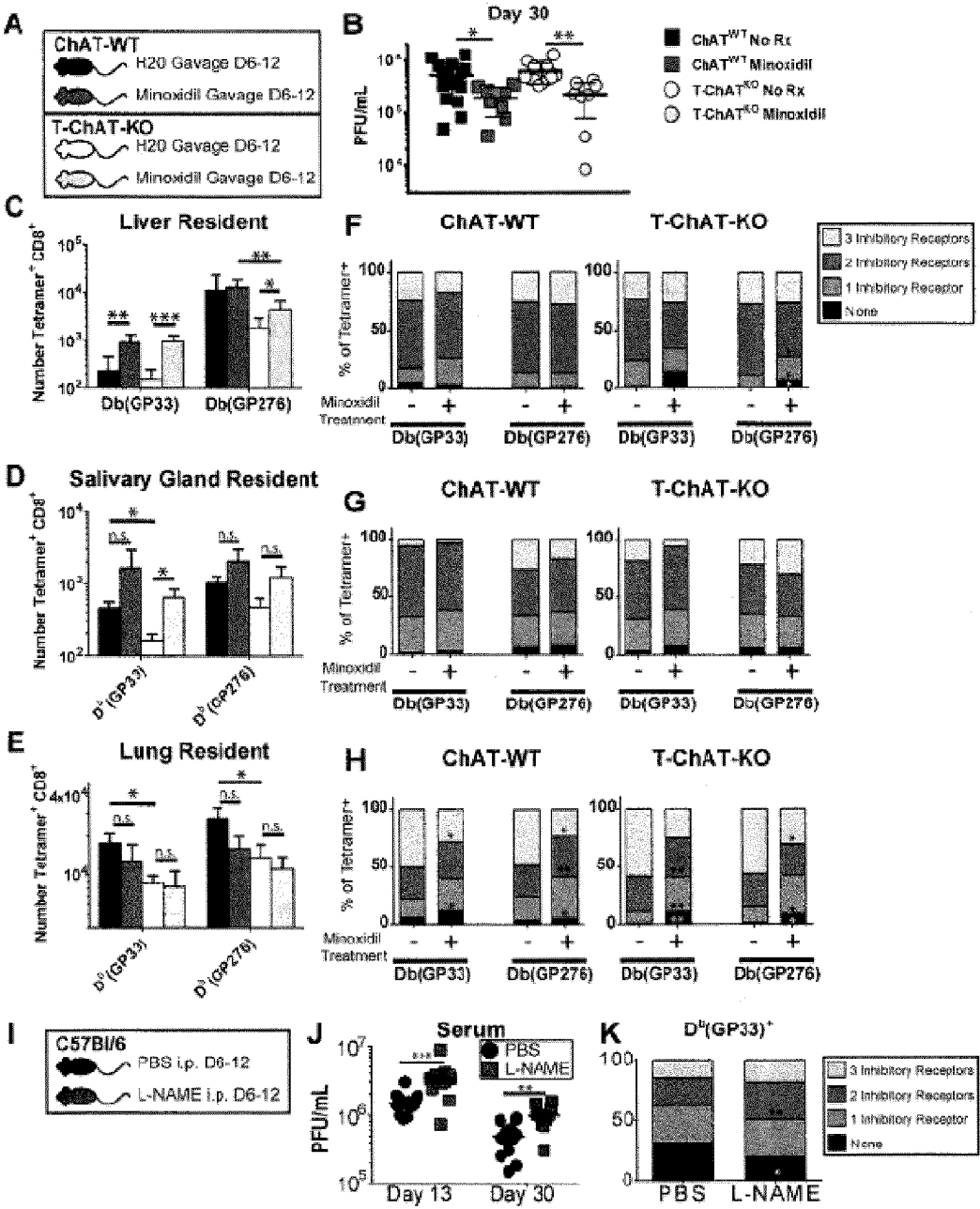
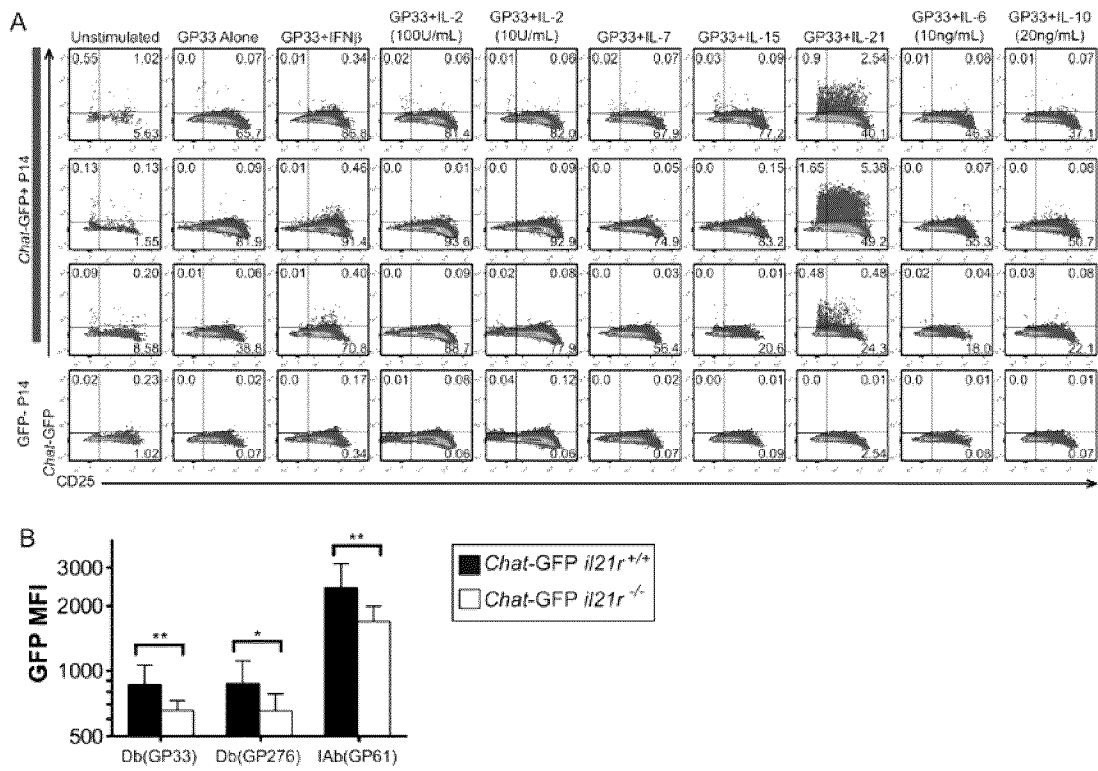


FIG. 8



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FIG. 9

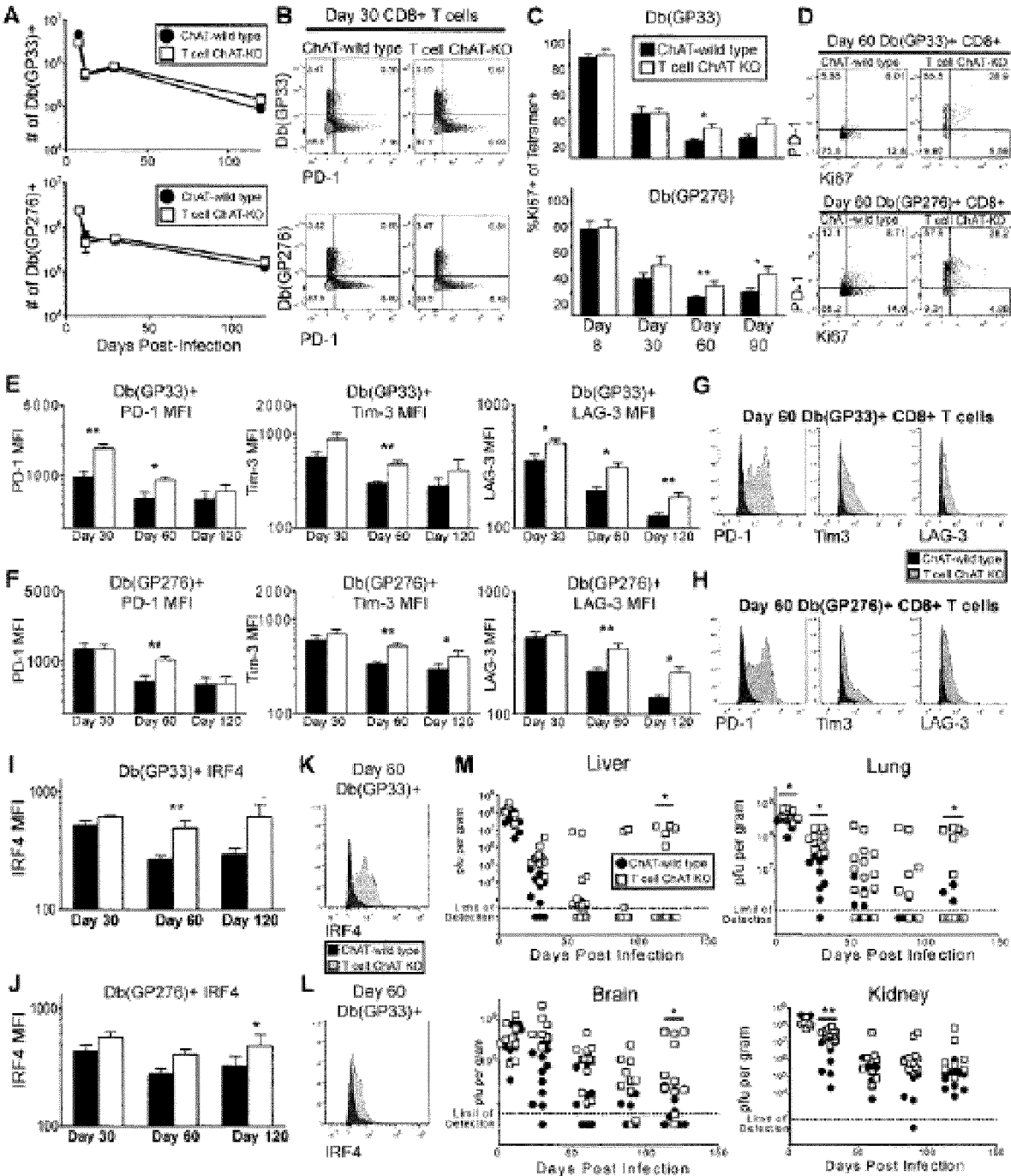


FIG. 10

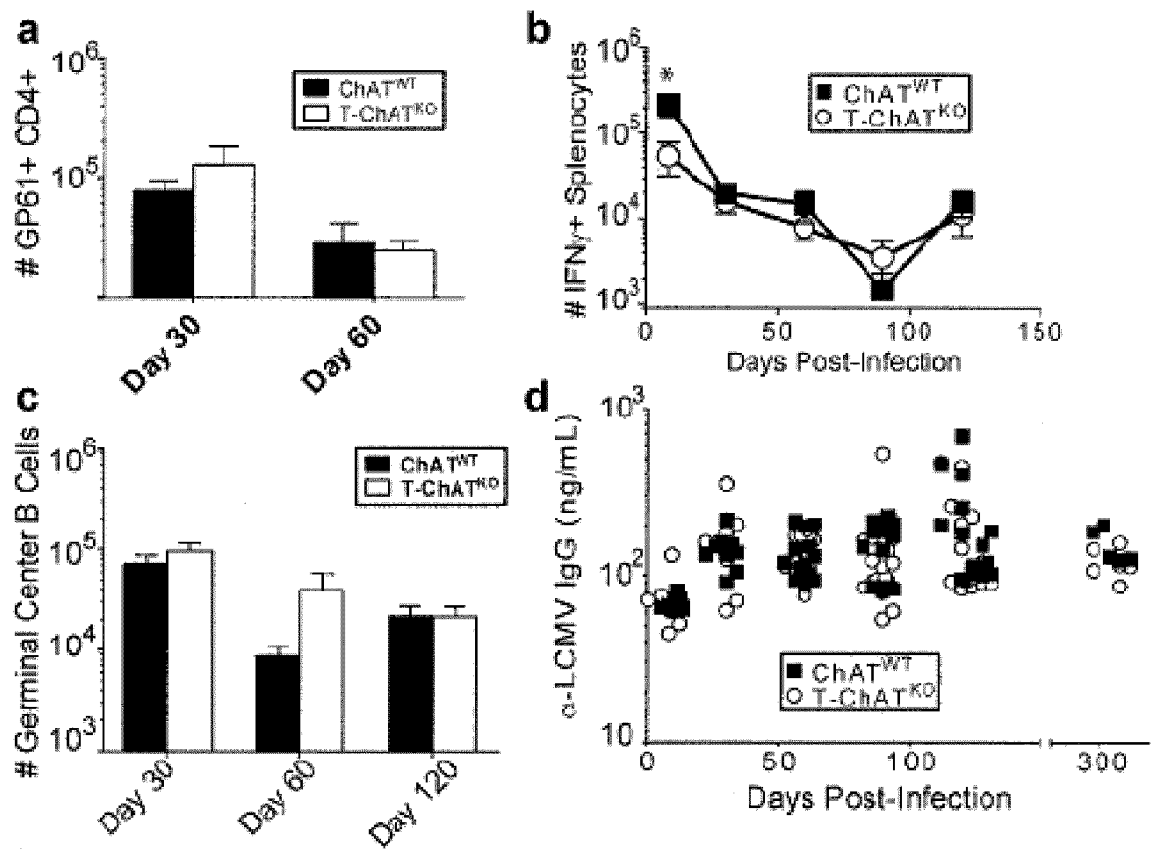


FIG. 11

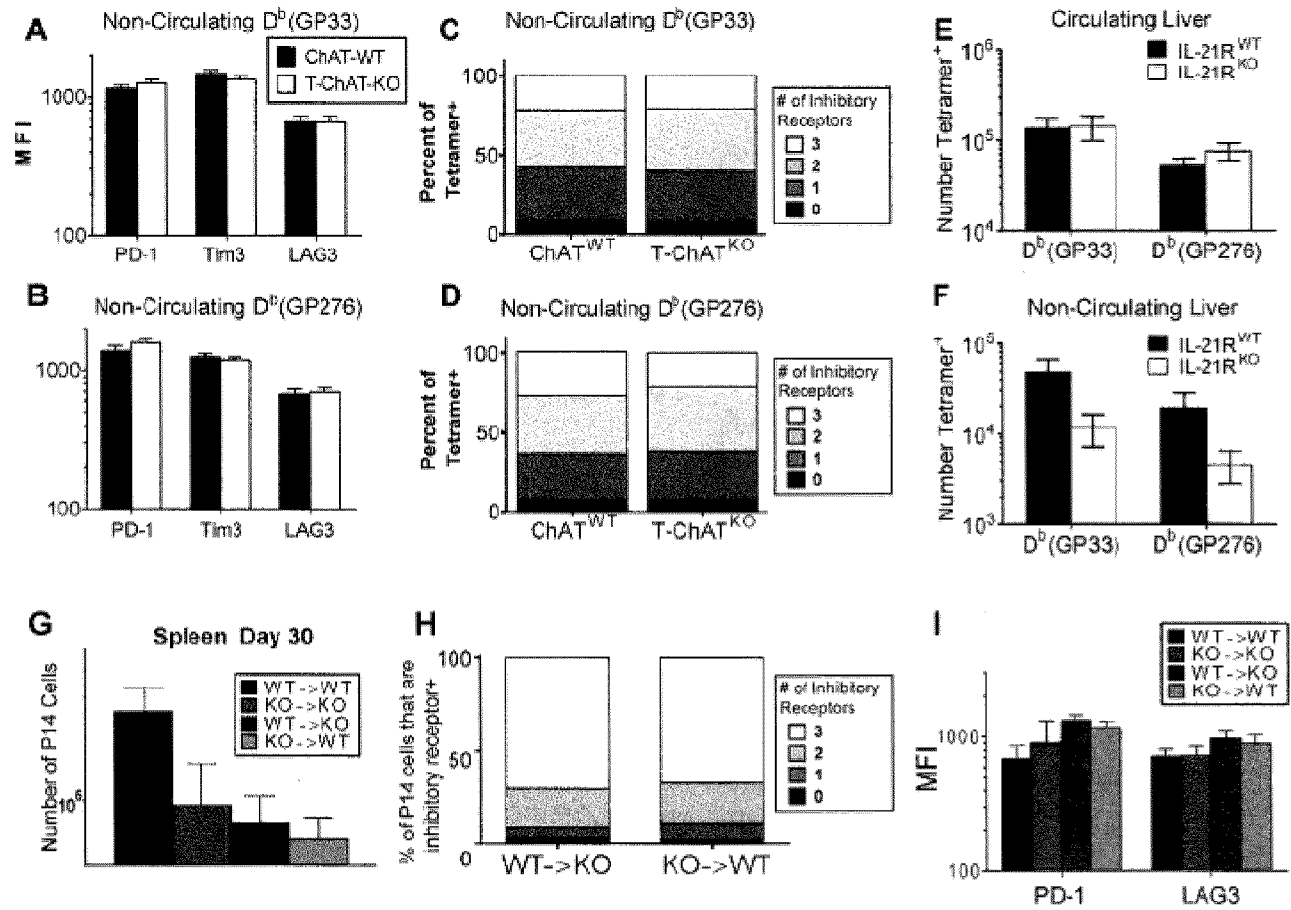


FIG. 12

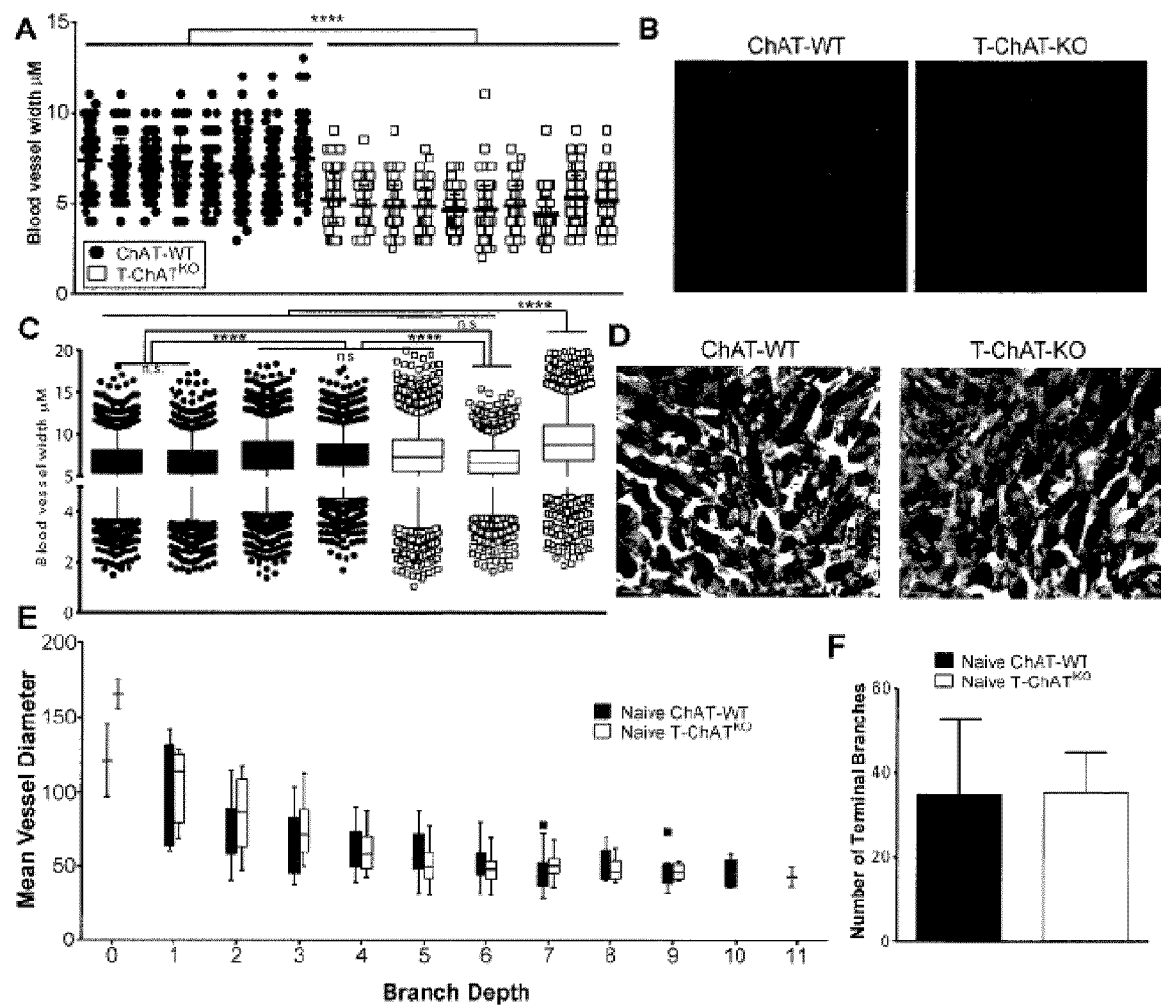


FIG. 13

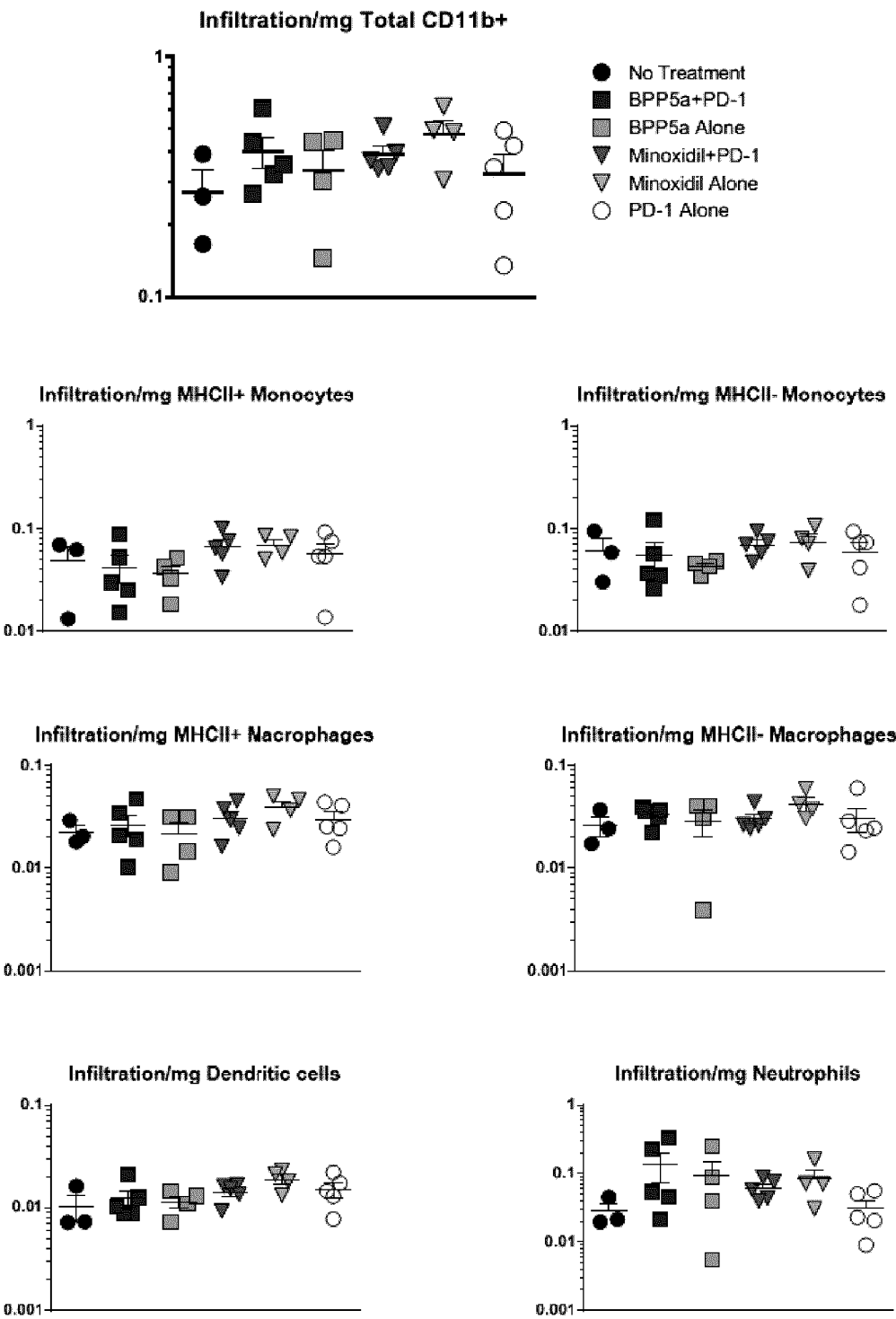


FIG. 14

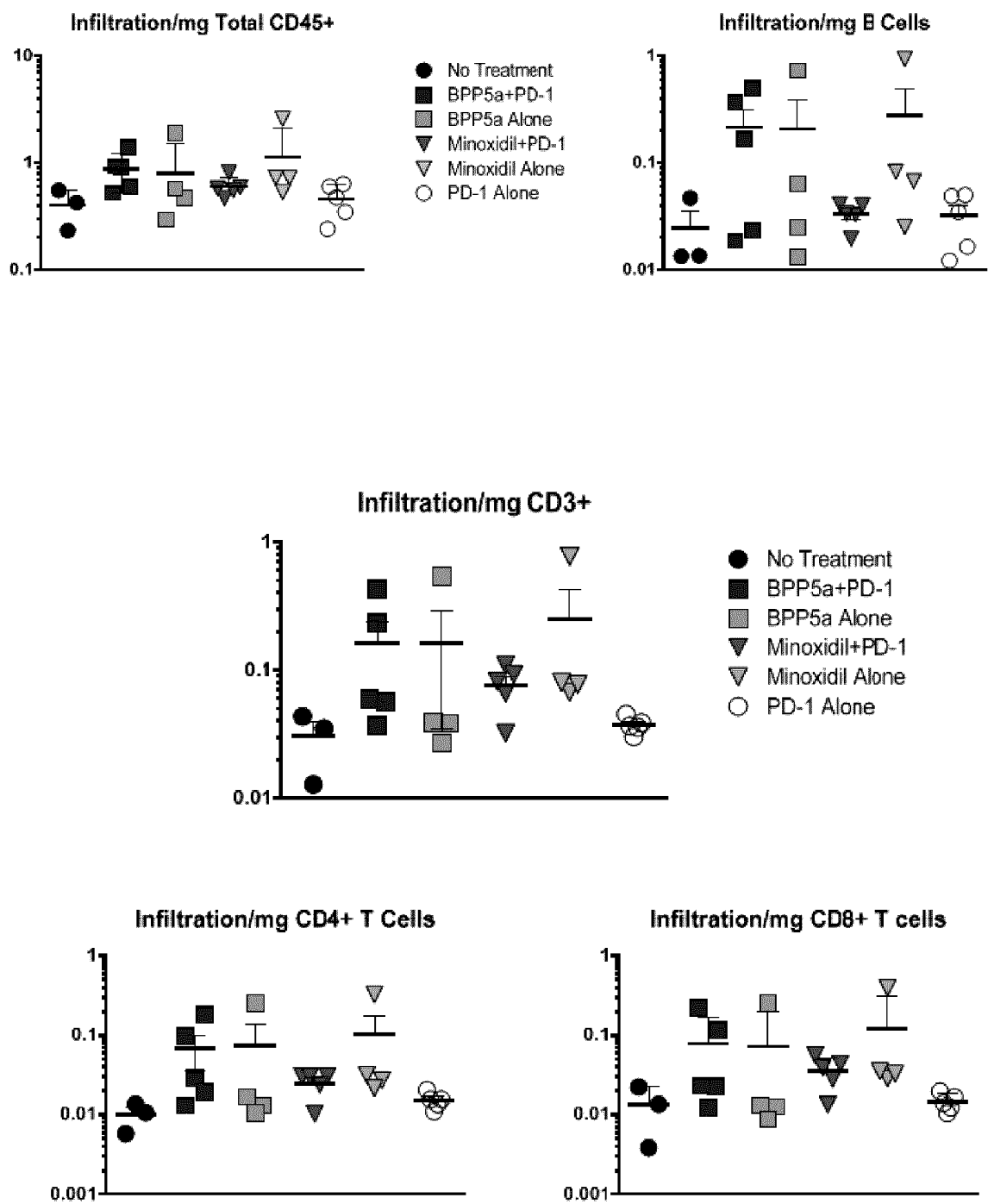


FIG. 15

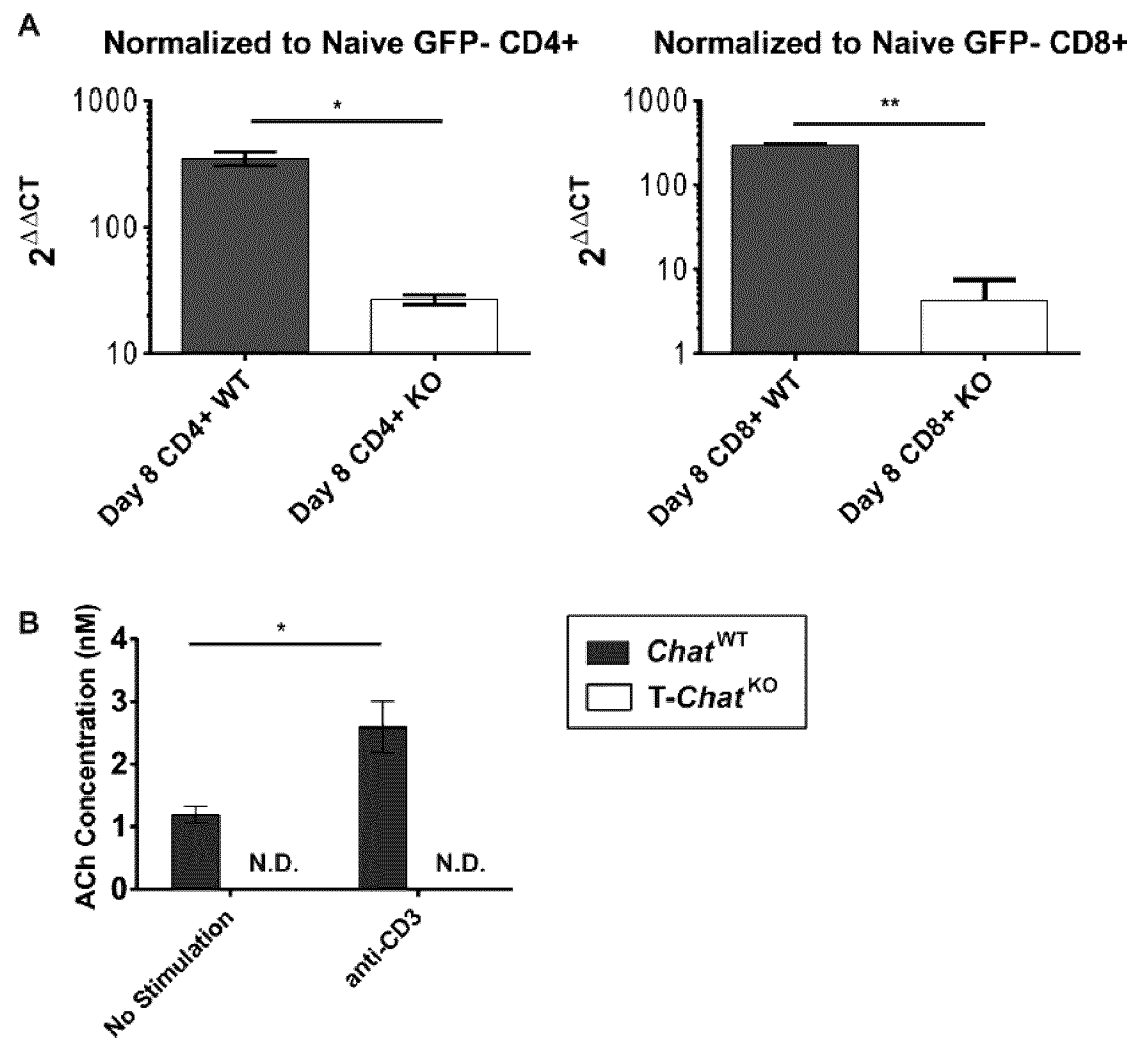


FIG. 16

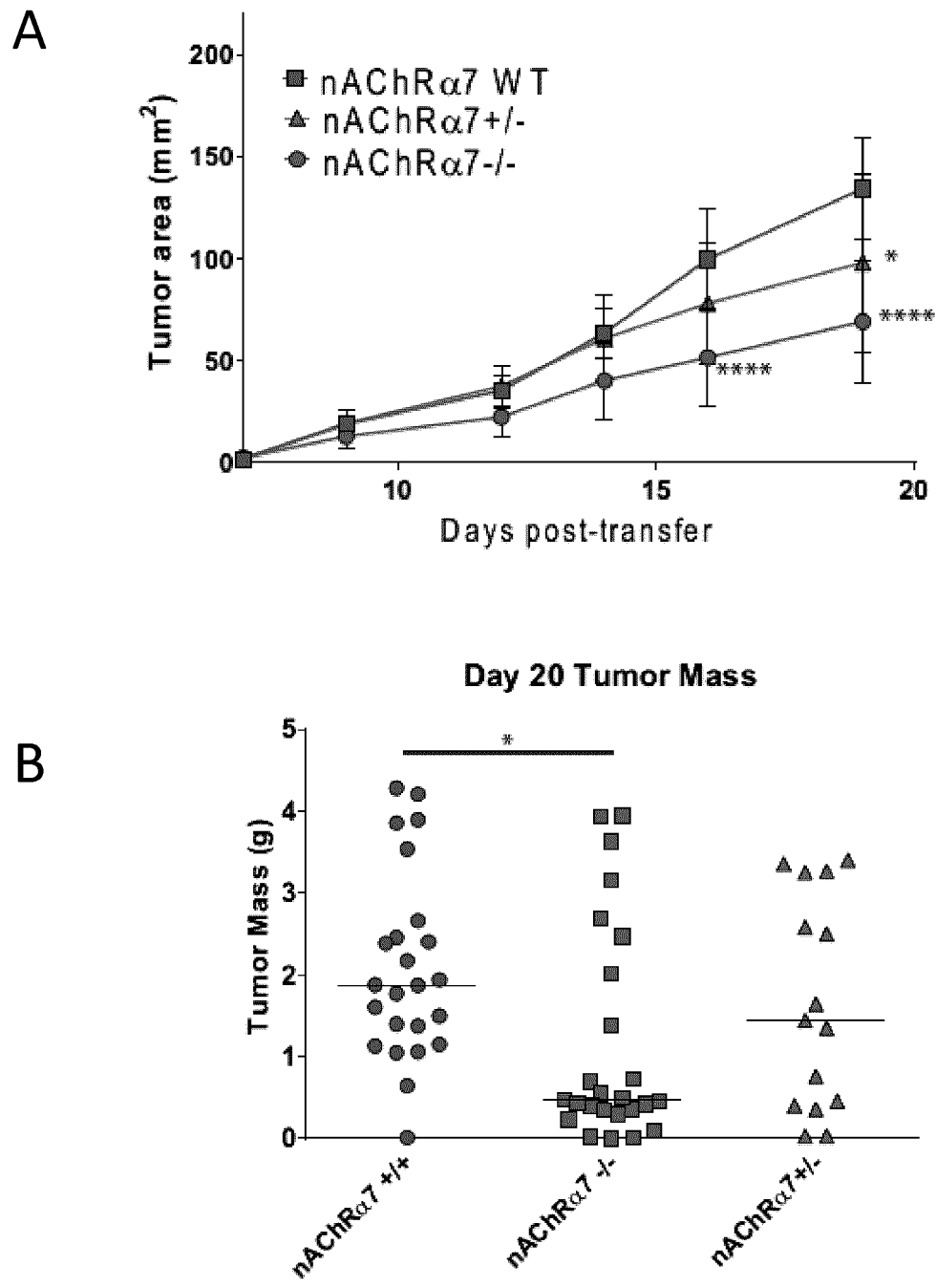


FIG. 17

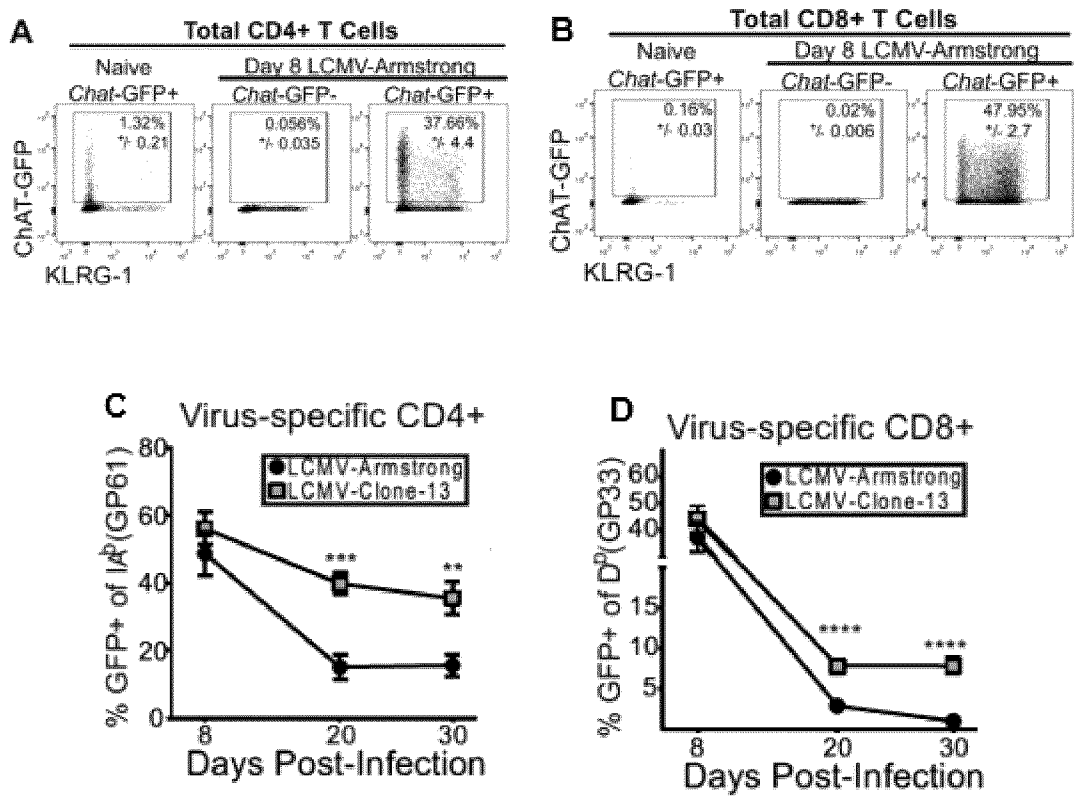


FIG. 18

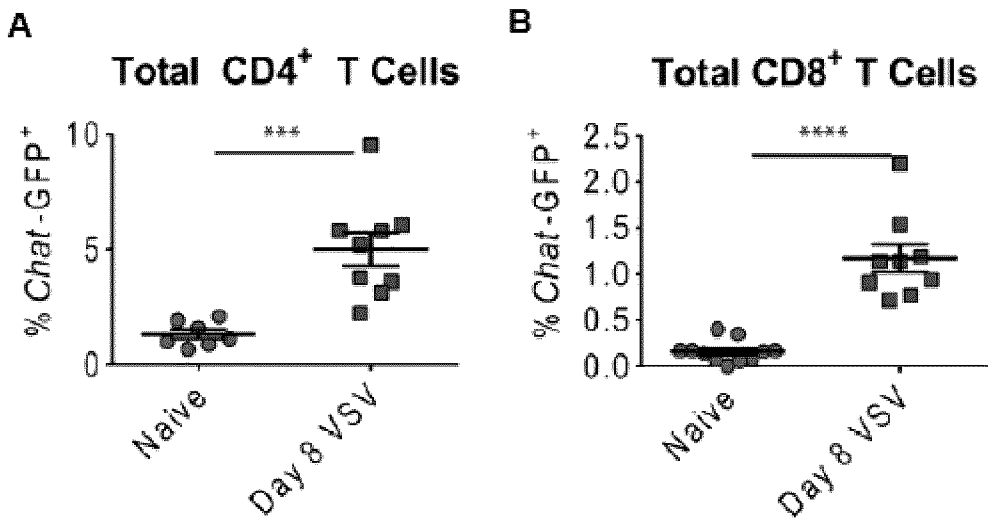


FIG. 19

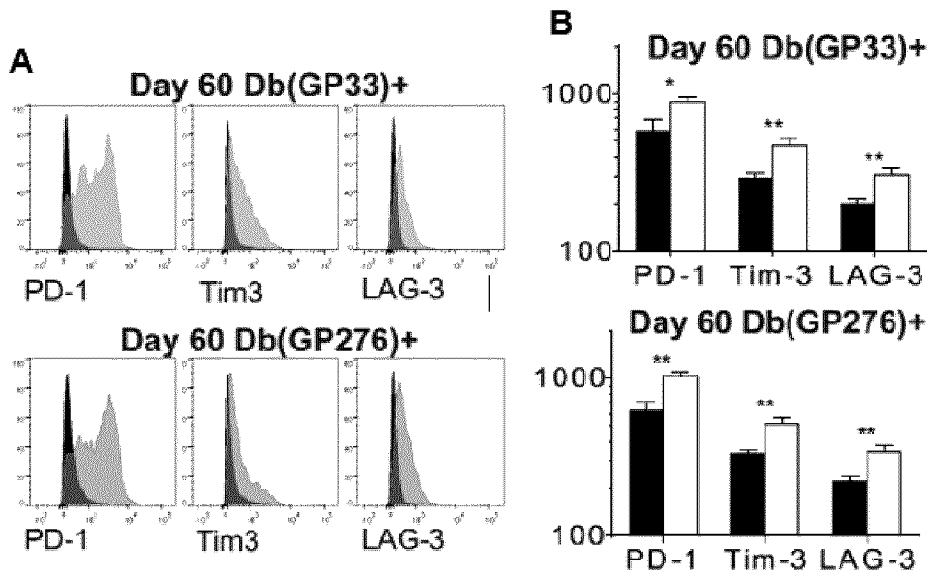


FIG. 20

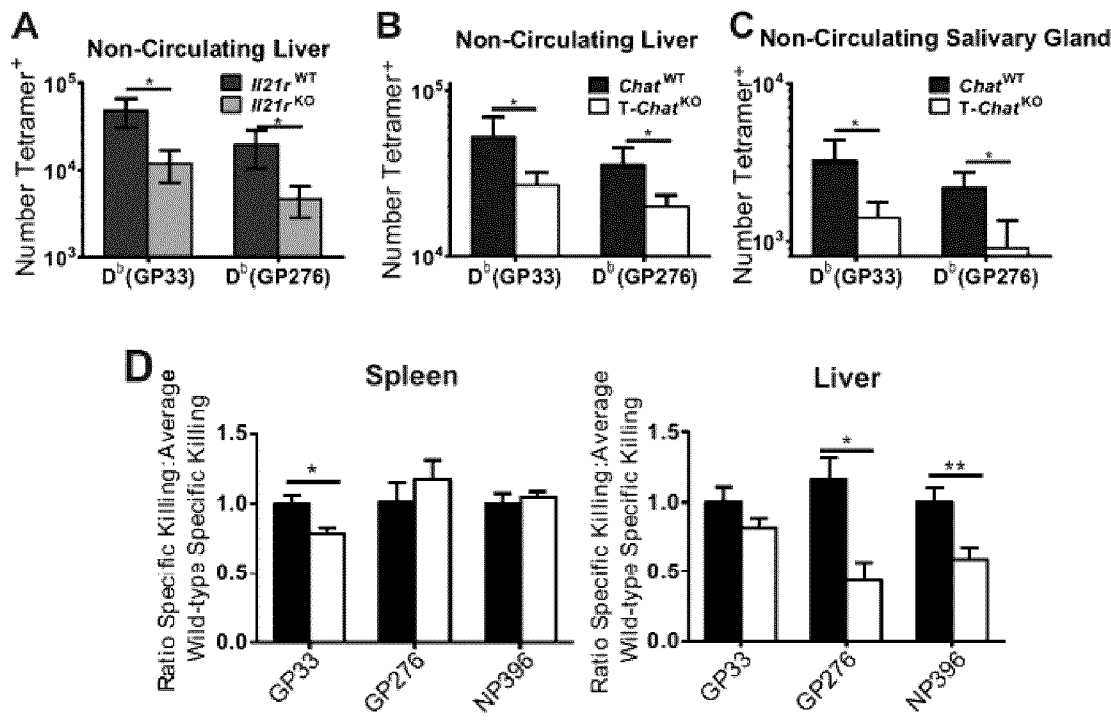


FIG. 21

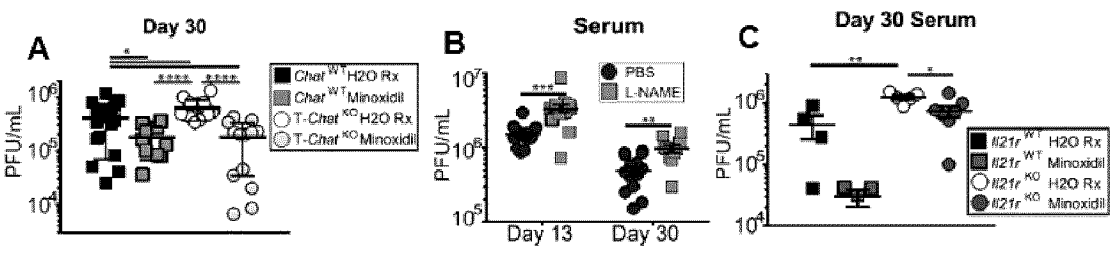


FIG. 22

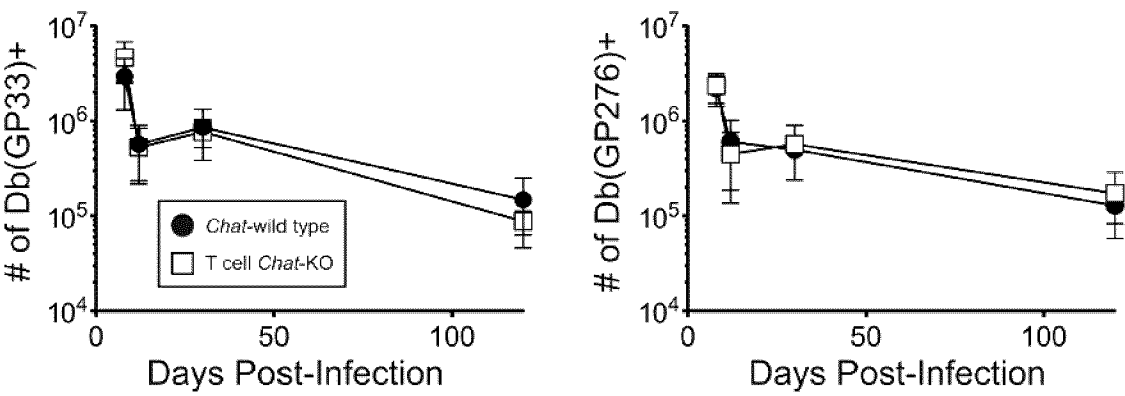
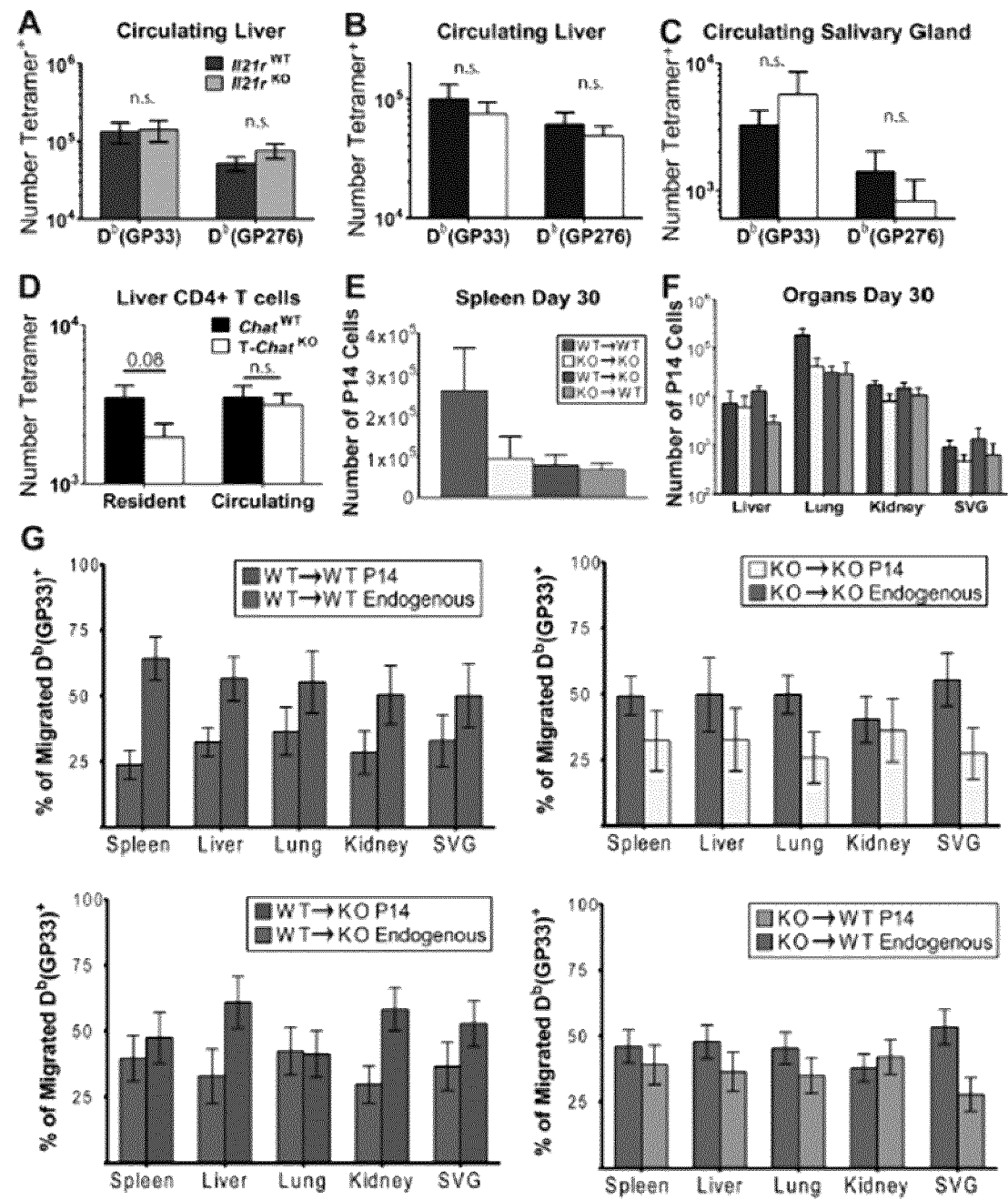


FIG. 23



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2020/050008

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 38/20** (2006.01), **A61K 35/17** (2015.01), **A61K 38/18** (2006.01), **A61K 47/55** (2017.01),
A61K 9/00 (2006.01), **A61P 31/12** (2006.01) (more IPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 All IPC searched

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases searched: Scopus, Google Scholar, Questel Orbit, and Canadian Patent Database
 Keywords: "choline acetyltransferase" AND ((cancer or tumor or neoplasm) OR (viral OR virus))
 Inventor and Applicant search in each above database.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	COX et al., " <i>Choline acetyltransferase-expressing T cells are required to control chronic viral infection</i> ". Science, 8 February 2019 (08-02-2019), Vol. 363(6427), pp. 639-44, ISSN 0036-8075	54-74
A	ROSAS-BALLINA et al., " <i>Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit</i> ". Science, 7 October 2011 (07-10-2011), Vol. 334(6052), pp. 98-101, ISSN 0036-8075	
A	REARDON et al., " <i>Lymphocyte-derived ACh regulates local innate but not adaptive immunity</i> ". Proc Natl Acad Sci U.S.A., 22 January 2013 (22-01-2013), Vol. 110(4), pp. 1410-5, ISSN 0027-8424	
A	FUJII et al., " <i>Physiological functions of the cholinergic system in immune cells</i> ". Front. Immunol., 6 September 2017 (06-09-2017), Vol. 8, pp. 1085, ISSN 1664-3224"	

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"D" document cited by the applicant in the international application	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"E" earlier application or patent but published on or after the international filing date	"&" document member of the same patent family
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
 10 April 2020 (10-04-2020)

Date of mailing of the international search report
 17 April 2020 (17-04-2020)

Name and mailing address of the ISA/CA
 Canadian Intellectual Property Office
 Place du Portage I, C114 - 1st Floor, Box PCT
 50 Victoria Street
 Gatineau, Quebec K1A 0C9
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Authorized officer

Michael O'Hare (819) 639-0581

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2020/050008

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CAMPOY et al., " <i>Cholinergic system and cell proliferation</i> ". Chemico-Biological Interactions, 25 November 2016 (25-11-2016), Vol. 259, PtB, pp. 257-65, ISSN 0097-2797	
A	KAWASHIMA and FUJII. " <i>Expression of non-neuronal acetylcholine in lymphocytes and its contribution to the regulation of immune function</i> " Front. Biosci., 1 September 2004 (01-09-2004)	
A	BECKMANN and LIPS. " <i>The non-neuronal cholinergic system in health and disease</i> ". Pharmacology, 29 November 2013 (29-11-2013), Vol. 92, No. 5-6, pp. 286-302, ISSN 0031-7012	

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The claims are directed to a plurality of inventive concepts as follows:

Group A - Claims 1-53 are directed to the use of choline acetyltransferase to treat cancer, or to the use of a vasodilator for treating a cancer having low level or activity of choline acetyltransferase; and

Group B - Claims 54-74 are directed to the use of choline acetyltransferase to treat viral infection, or to the use of a vasodilator for treating a viral infection associated with low level or activity of choline acetyltransferase.

Choline acetyltransferase is well known in the art and so cannot serve as a unifying inventive concept.

The claims must be limited to one inventive concept as set out in PCT Rule 13.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

A61P 35/00 (2006.01), *C07K 14/52* (2006.01), *C07K 16/40* (2006.01), *C12N 5/0783* (2010.01),
C12N 9/10 (2006.01), *C12Q 1/48* (2006.01), *C12Q 1/6809* (2018.01), *G01N 33/574* (2006.01),
C12N 15/54 (2006.01), *G01N 33/48* (2006.01)